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Enhancement of Membrane Performance via Biofilm Management

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Enhancement of Membrane Performance via Biofilm Management

A dissertation submitted in partial satisfaction of the

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in Civil Engineering

by

Caroline Kim

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ABSTRACT OF THE DISSERTATION

Enhancement of Membrane Performance via Biofilm Management

by

Caroline Kim

Doctor of Philosophy in Civil Engineering

University of California, Los Angeles, 2019

Professor David Jassby, Chair

Biofilm formation is a concerning issue for many industrial processes, especially for water treatment membranes, because it leads to fouling and poor performance of the system. Combinations of physical and chemical cleaning methods are commonly used to remove the deposited biofilms. However, there is little understanding of the interactions between chemical cleaning agents and the biofilm. Therefore, companies perform a series of iterations whereby a certain cleaning product or process is applied to the surface with the hope of dislodging the biofilm. This leads to indiscriminate use of cleaning agents and inconsistent results of biofilm removal. The

goal of this project is to give insights to the impact of representative cleaning agents on individual biofilm components and ultimately, to design an efficient microbial cleaning strategy for biofouled surfaces, which will help to ensure a safe and sustainable operation of water supply and enhance the performance of membrane-based water treatment processes.

In this study, the interaction between a homogeneous layer of a single biofilm component (polysaccharides, proteins, nucleic acids) and different cleaning solutions (base, oxidizer, surfactant, chelating agent) was evaluated by comparing permeate flux of an ultrafiltration membrane and frequency shift measured by a quartz crystal microbalance. The efficacy of cleaning agents towards model biofilm component mixtures designed to mimic gram-negative and gram-positive bacterial biofilms and an actual bacterial biofilm component extracted from *Pseudomonas aeruginosa* (a gram-negative bacteria) were tested. The presence of calcium in the feed solution hindered the ability of the cleaning solutions to completely remove the foulants except when a chelating agent was used. The usage of a base and an oxidizer showed the best performance yet did not fully removed the model biofilm component mixtures or the bacterial biofilm component. Overall, it was determined that the presence of proteins in biofilms determines their susceptibility to cleaning.

The dissertation of Caroline Kim is approved.

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Chapter I.

Introduction

1.1 Advanced Water Treatment: Membrane Filtration

Membrane filtration is one of the fastest growing advanced water treatment processes. Growing demand for high quality water and reuse of wastewater brought filtration membranes to the spotlight and impacted the global membrane market. The global membrane market for water treatment and industrial uses combined grew from $19.0 billion (in 2010) to $21.2 billion within 3 years[1]. According to SBI Energy report, reverse osmosis (RO) membrane alone will reach $40 billion by 2020 – contributing to the largest growth in the global membrane and desalination market[2]. Membrane filtration is a promising to solution to global water shortage due to its various advantages over conventional water treatment processes.

Unlike conventional water treatment technologies, including coagulation, sedimentation, distillation, and media filtration, membrane filtration does not require additional chemicals, thermal input, or replacement/recharge of used media.[3] The system is also more reliable and easier to operate at a lower maintenance and operating costs. Moreover, the smaller infrastructure and high efficiency of water produced/water fed ratio of the system are compelling reasons to implement membrane filtration in land-scarce cities and countries. Above all, the process can offer varying degrees of salt selectivity to achieve specific water quality level whereas conventional water treatment can only remove contaminants with sizes > 1μm.[4]

Implementation of membrane filtration has widely spread all around the globe to provide safe water to the community. California, the most populated state in the United States, provides water to almost 40 million people[5] using various types of water treatment processes, including membrane filtration. One of the popular membrane filtration facilities in Southern California is in Orange County Water District (OCWD), which has the world's largest advanced water purification system for portable reuse that utilizes filtration membrane. It produces 100 million gallons of water

per day (MGD) to 850,000 residents in the county and expects to expand its capacity to 130 MGD by 2023[6]. Another example is Advanced Water Purification Facility (AWPF) created by Terminal Island Water Reclamation Plant (TIWRP) and Los Angeles Department of Water and Power (LADWP). This facility, located in San Pedro, provides 12 MGD of recycled water to the Dominguez Gap Barrier (DGB) using membrane filtration and advanced oxidization process[7]. Meanwhile, early 2019, the Mayor of L.A. has announced to recycle 100% of wastewater at a different facility, Hyperion Water Reclamation Plant (HWRP) by 2035[8]. Currently the facility receives 81% of the city's total wastewater (average of 275 MGD) and recycles only 27% of that water coming into the facility[9]; almost 75% of the incoming wastewater is being discharged to the ocean. According to the report from Bureau of Reclamation, the City of L.A. proposed, in 2016, a project of implementing advanced water treatment, which comprises membrane bioreactors, reverse osmosis and advanced oxidation process, to produce more reclaimed water at HWRP. Therefore, there is a high possibility that HWRP will utilize the membrane filtration process in order to achieve 100% wastewater recycle rate.[10] Clearly implementation of membrane filtration has widely spread and is expected to continually grow.

Membrane filtration has emerged as the leading technology in water treatment processes and may continue to be of critical importance to alleviate stresses on the global water supplies. There is growing interest in modifying the current filtration membranes and the future is likely to see the continued development of high performing membranes with enhanced water productivity and contaminant (especially, salt) removal. In this chapter, we address the membrane filtration mechanisms, challenges and new techniques that are gaining interest in the field.

1.2 Basics of Membrane Filtration

According to the European Membrane Society, a membrane is defined as an intervening phase separating two phases and/or acting as an active or passive barrier against the transport of matter between phases.[11] In essence, it is a thin, semi-permeable layer, which separates two phases (e.g. undesirable constituents and water) by a driving force: a gradient of either pressure, concentration, electrical potential, temperature, gravity, vacuum or etc.[12]

The optimal goal for membrane filtration or any water treatment process is to maximize the production of clean water and effectively remove contaminants with low capital and operating costs. Most membrane filtration for water treatment process utilizes pressure as the driving force. The productivity of the pressure-driven membrane filtration process is often characterized by an operating parameter called flux, which is defined as the filtrate volumetric flow (Q) per unit of membrane area (A). Using modified Darcy's law, water flux can also be expressed in terms of pressure (driving force), as shown in Eq (1).[13]

$$J_w = \frac{Q}{A} = \frac{(\Delta P - \Delta \pi)}{\mu R_m} \quad (1)$$

To produce more water or to increase the water flux, a sufficient amount of hydraulic pressure (ΔP) must be exerted to overcome the osmotic pressure ($\Delta \pi$) difference across the membrane. Note that the flux is highly influenced by operational conditions, such as applied hydraulic pressure, cross-flow velocity, water temperature and water viscosity (μ). The hydraulic resistance of the membrane to water permeation (R_m) incorporates the resistance attributed from the intrinsic membrane property and fouling − deposition and accumulation of undesirable materials − that inhibits the transport of water across the membrane. The rate and severity of fouling may depend on the type or amount of contaminants in the water and operational condition.

Thus, in order to maximize the water production or water flux, optimizing the operational conditions of the filtration process is crucial.

For instance, to effectively remove contaminants, it is important to choose the appropriate separation mechanisms or membrane types for the water treatment process. There are four different types of filtration membranes: Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO).[4,14,15] MF and UF membrane use the sieving mechanism, which removes pollutants based on the size of the membrane pores. For NF and RO, separation is achieved by the difference in diffusion rates of water and contaminants through the membrane, also known as the solution-diffusion mechanism. Typically, the membrane type for the water treatment process is chosen based on the size of the contaminants in the water (Figure 1.1).

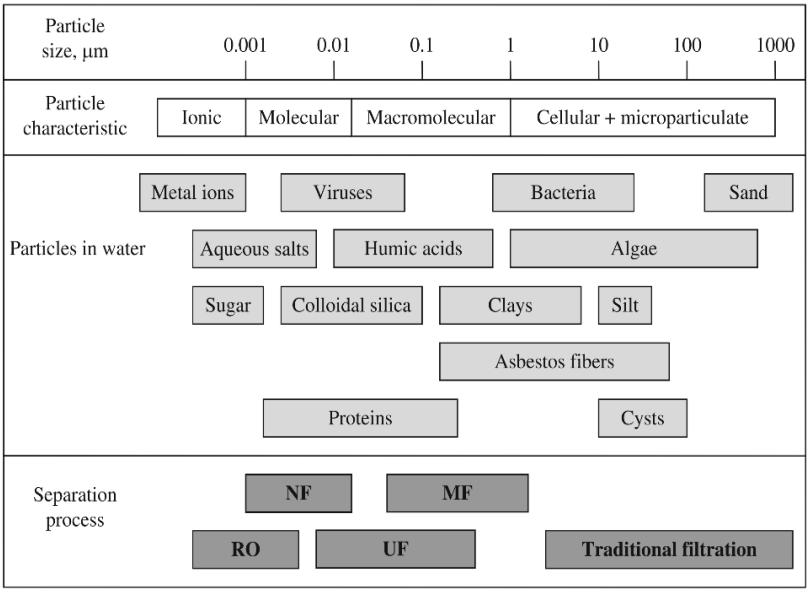

Figure 1.1 Application range of various membrane processes[4]

The MF membrane has the biggest pore size, ranging from 0.1 - 10 μm, and operates at low pressure (1 - 2 bars) compared to other filtration membranes. It is best known for separating suspended particles and large colloids including bacteria and algae[4]. UF is also a low pressure

driven process (2 - 10 bars) but has smaller membrane pore sizes than the MF membrane, which enables the removal of smaller contaminants like viruses and proteins. The UF membrane has a pore size ranging from 0.01 - 0.05 mm but is more commonly characterized by the molecular weight cutoff (MWCO), the molecular weight at which 90% of the solute is rejected by the membrane. Typical UF MWCO is 10,000 - 500,000 Daltons.[16] Unlike MF and UF membranes, NF and RO membranes have a nonporous, dense structure, which serves as a barrier for metal or salt ions but requires high pressure to operate (10 - 70 bars). RO is generally used for desalination of seawater or brackish water because of its high salt rejection rate (most RO membrane has 96 - 99 % NaCl rejection) and is relatively less energy-intensive compared to other desalination processes (e.g. distillation) since it involves no phase change. NF is referred to as "loose" RO or "tight" UF due to its excellent capability of removing multivalent ions but poor rejection of monovalent ions. Moreover, it requires less pressure (7 - 14 bars) and energy than RO to produce filtered water at a higher flux.

There are two ways to operate these membrane filtration processes: dead-end and cross-flow filtration.[11,12] In dead-end filtration, the entire solution (feed stream) is forced through the membrane (Figure 1.2A). In cross-flow filtration, the feed stream flows tangentially to the membrane surface and only a portion of the feed stream passes through the membranes under pressure (Figure 1.2B). The filtered water that is passed through the membrane is defined as the permeate stream, and the concentrated solution retained on the feed side of the membrane is called the retentate stream. Since there is no retentate stream for dead-end filtration, it has a higher recovery of concentrated feed and simpler operation compared to cross-flow filtration. The continuous deposition of substances on the membrane surface, however, leads to an increase in the hydraulic resistance and thus decrease in permeate flux over time. Therefore, the membrane must

be replaced frequently, or an additional step is required (e.g. back-flushing) to remove the concentrated matter in dead-end filtration. This mode is recommended for slightly contaminated solutions or processes for concentrating compounds. Cross-flow filtration is preferable over dead-end filtration since the tangential flow scours away contaminants from the membrane surface and limits the accumulation of the contaminant, which may prolong the membrane life-span. However, the operating cost of the cross-flow filtration is higher than the dead-end filtration because of the energy needed to circulate the feed stream. Depending on the application, either operational mode can be used in water treatment processes.

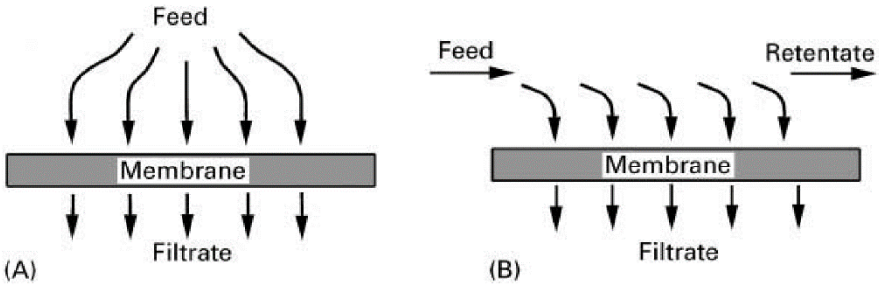

Figure 1.2 Two modes of membrane filtration (A) dead-end filtration (B) cross-flow filtration

1.3 Challenges of Membrane Filtration

An ideal filtration membrane should have the following characteristics[12]: 1) high flux, 2) high salt rejection, 3) mechanical and chemical stability, 4) fouling resistance 5) tolerance to variations in temperature, pH and operating pressure, and 6) low manufacturing cost.[17] Unfortunately, membrane that satisfies all these characteristics is not commercially available. Any advancement in improving the membrane characteristics listed above would ensure a sustainable industrial growth of existing membrane filtration processes and promote usage of filtration membranes for new applications.

While current membrane filtration processes perform well in many water treatment applications, they are subject to certain limitations such as, poor rejection of selective dissolved or uncharged ions (e.g. boron), high energy consumption for desalination, and disposal of concentrated waster/brine.[3] Among the challenges that membrane filtration processes face, the main concern is the loss of filtration performance (flux decline) due to concentration polarization and membrane fouling[4,15,18,19], which leads to additional costs for membrane cleaning and replacement.

1.3.1 Concentration Polarization

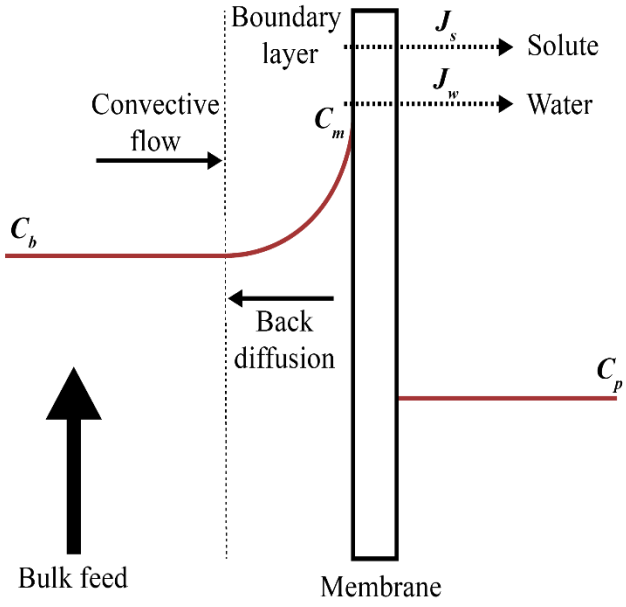

Figure 1.3 Concentration Polarization

All membrane separation processes encounter a phenomenon called 'concentration polarization' in which rejected solutes accumulate near the membrane surface. During filtration, the imposing transmembrane pressure transports the solute convectively towards the membrane surface. The rejected solute accumulates on the membrane surface, and the solute concentration at the membrane surface therefore gradually increases. As a result of the concentration gradient between the membrane surface and the feed bulk, the solute diffuses back into the feed solution. At steady state, the convection of solutes toward the membrane surface is balanced by the diffusion away from the membrane surface, creating a polarization boundary layer (Figure 1.3). Due to the high solute concentration at the membrane surface, the effective osmotic pressure at the membrane-solution interface is elevated and the required transmembrane pressure

for operation is increased. This ultimately leads to flux decline and promotes fouling. Concentration polarization also increases the solute permeation through the membrane because of the increase in the transmembrane concentration gradient generated. Therefore, it is important to minimize the effects of concentration polarization during the membrane filtration process.

The primary method of minimizing concentration polarization is to reduce the thickness of polarization boundary layer by increasing turbulent mixing at the membrane surface. The most direct method is to increase the fluid flow velocity, although it would increase the operating cost. More efficient approach to promote turbulent mixing is to implement a feed-spacer.[20] Also, employing cross-flow filtration instead of dead-end filtration can reduce but not completely eliminate concentration polarization.[12]

1.3.2 Membrane Fouling

In addition to concentration polarization, the second cause of flux decline is membrane fouling. Membrane fouling is defined as the deposition and accumulation of undesirable materials, also known as foulants, on or within the membrane structure. Accumulation of foulant manifests with time and gradually results in an irreversible loss of the water permeability under constant pressure. Typically, membrane fouling accounts for 30% of membrane system operation cost due to increased energy demand, membrane cleaning, membrane replacement and additional labor cost for maintenance.[21] Therefore, membrane fouling is an inevitable and costly problem for all membrane filtration processes.

According to the nature of foulants, membrane fouling can be commonly classified as scaling (inorganic fouling), organic fouling, colloidal fouling and biofouling.[19,22–24]

1.3.2.1 Scaling (Inorganic Fouling)

Scaling occurs when sparingly soluble mineral salts concentrate beyond their solubility limits, due to the loss of water through permeation, and precipitate to form an impermeable layer on the membrane surface. Minerals that precipitate and form scaling are predominantly multivalent metal ions such as calcium, iron, magnesium, and aluminum because they are almost insoluble in the presence of carbonate, sulphate, phosphate and hydroxide ions[23]. The most common scale found in membrane filtration for water treatment is calcium carbonate because calcium and carbonate ions are ubiquitous in natural waters and their low solubility in water allows the mineral to precipitate quickly. Scaling can be minimized by lowering the pH, adding an anti-scalant, reducing product water recovery, preventive cleaning, or a combination of all these techniques[25]. The best practice is to operate the system below the critical solubility limits of the minerals, but there is no reliable way to fully predict the water chemistry and physical condition of the feed stream.

1.3.2.2 Organic Fouling

Organic fouling is caused by the deposition of organic substances including proteins, polysaccharides, humic acids and natural organic matter (NOM). Hydrophobic membranes are usually more prone to organic fouling because most organic matters in the feed water are hydrophobic and preferentially tend to adsorb onto the membrane surfaces. The metal cations (e.g. calcium ions) present in natural waters can cross-link and form bridges between negatively charged functional groups of organic matters and/or negatively charged membrane surface. This interaction reduces the charge and electrostatic repulsion of the organic matter and can lead to rapid fouling on the membrane, forming a dense gel layer. Organic fouling can be reduced by adding a strong

chelating agent (e.g. EDTA), which can remove free and bound metal cations and loosen the densely packed fouling layer, in the feed water.[26]

1.3.2.3 Colloidal Fouling

Commonly found colloidal particles in natural water are clays, silica, iron and aluminum hydroxides, and organic debris, with size ranging from a few nanometers to a few micrometers. During membrane filtration, colloidal particles aggregate on the membrane surface or within the membrane pores causing colloidal fouling. The rate of colloidal fouling is influenced by permeation drag force, electrostatic repulsion, solution ionic strength, feed colloid concentration, membrane surface roughness, and membrane surface charge.[27,28] Colloidal fouling can enhance concentration polarization by hindering the back diffusion of rejected salt ions and cause significant flux decline.[29] Therefore, it is recommended to lower the feed pH to prevent negatively charged colloidal particles to coagulate or physically remove the fouled layer by air scrubbing or backwashing.[30]

1.3.2.4 Biofouling

Biofouling is caused by the microorganisms or bacteria that can grow to form a biofilm. Biofilm is first developed by the conditioning layer settled onto the surface which is composed of particles, organic or inorganic present in the bulk fluid.[31] This layer modifies the surface to facilitate the bacteria attachment. The attachment is influenced by forces including van der Waals attraction, electrostatic repulsion and hydrophobic interactions.[32] Over time the reversibly attached bacteria will become irreversibly adsorbed onto the surface and will rapidly grow at the expense of surrounding nutrients from the feed. Then the bacteria start excreting extracellular polymeric substances (EPS) that establish the functional and structural integrity of the biofilm.[33] Once the biofilm fully matures, the cell breakdowns to release surface bacteria which disperse into the

environment and grow more biofilm in other areas, making it almost impossible to completely remove biofilm from the system.

In most biofilms, the microorganisms account for less than 10% of the dry mass, whereas the EPS can account for over 90%.[33] Therefore, EPS dominates most of the composition of the biofilm. Since the EPS is mostly produced by the bacteria themselves, it is composed of the same molecular species, including polysaccharides, proteins, nucleic acids, and lipids. These components are responsible for binding the bacteria to the surface and protecting the bacteria from the surrounding environment by serving as a physical barrier and nutrient source. Therefore, elimination of biofilm is a complicated task and it is important to completely remove the microbial cells and deposited biofilm from the surface to prevent rapid re-fouling.

1.3.2.5 Fouling Mechanism

Membrane fouling is a very complex phenomenon, which is the result of multiple forces between feed components and the membrane surface including fluid drag, van der Waals, electrostatic, or hydrophobic/hydrophilic forces. Therefore, the nature and extent of fouling is strongly influenced by the feed solution properties (concentration, pH, ionic strength and component interactions), membrane characterization (hydrophobicity, charge, roughness, pore size, pore size distribution and porosity) and operating conditions (temperature, transmembrane pressure (TMP) and cross-flow velocity).[24] Under these forces, fouling can occur via three different mechanisms: pore adsorption, pore blocking, and cake formation.[20,34] While pore adsorption and blocking are known as internal fouling mechanisms, cake formation occurring on the membrane surface is defined as external fouling mechanism.

Pore adsorption occurs when foulants smaller than the membrane pore size is deposited on the pore walls along their entire length. Adsorption is generally driven by either or combination of weak van der Waal forces, electrostatic attraction, or chemical bonding, depending on the functional groups of the foulant and membrane.[34] Even in the absence of a permeation flux, it can occur spontaneously and almost instantaneously. The decrease in membrane pore diameter or volume due to the adsorbed foulant on the wall results in permeate flux decline, although the overall number of pores remains constant.

Meanwhile, pore blocking causes flux decline by full or partial closure of the membrane pores. The pore diameter remains the same, but the pore volume decreases with the reduction in total number of pores. Pore blocking usually happens in the initial stages of filtration when foulants are in direct contact with the bare membrane surface/pore. While the cause of pore volume reduction differs between pore adsorption and blocking, both mechanisms cause reduction in membrane permeability.

The cake formation occurs when foulants larger than the membrane pore size builds up layer by layer on the external surface of a membrane, leading to an additional resistance, also known as “cake resistance”, to the permeate flux. When the first cake layer is formed by inert foulants, it prevents active foulants from directly interacting with the membrane surface. This layer acts as a prefilter, referred as “filter-aid”, and can be easily removed using high cross-velocity flow or backwashing because it is reversibly attached on the membrane surface. However, the filter-aid rarely exists in natural environments. When the active and inert foulants randomly mix together, it forms a more adhesive cake layer, which is irreversibly attached to the surface and harder to remove. Sometimes, ‘over-clogging’ may take place, where small foulants penetrate or fill the gap

in the cake layer, clogging the pores even further and increasing the cake layer density and hydraulic resistance of the membrane.

1.4 Strategies for Managing Fouled Membrane

The development of fouling resistant membrane surfaces or pretreatment system has been an ongoing research objective for several decades. To date, there are no strategies employed in the membrane filtration processes to completely prevent fouling at the commercial scale. Therefore, periodic membrane cleaning is necessary to maintain the membrane performance. Membrane cleaning is a direct strategy to alleviate membrane fouling by restoring the water permeability.

Typically, cleaning is performed when one of the following conditions is met[4]: 1) 10 % decrease in water production at constant operating conditions, 2) 10 % increase in the driving pressure to maintain the same production at constant temperature and, 3) an increase of 15 – 20 % in the pressure differential between feed and reject flows. Membrane cleaning is largely categorized into two methods, physical and chemical cleaning, based on the fouling removal mechanisms or cleaning agents used.

1.4.1 Physical cleaning

Physical cleaning generally involves hydraulic or mechanical cleaning to loosen and dislodge the reversibly attached foulants.[23,34,35] Backwashing is the most common hydraulic cleaning method, which uses the reversed flow to remove the foulants attached to the membrane pores or surface. Implementing intermittent operation (membrane relaxation) of the membrane filtration is the simplest method to remove fouling, which allows concentrated foulants at the membrane surface to diffuse away driven by the concentration gradient. For mechanical approaches, air scouring or using a spacer can promote higher turbulence at the membrane surface and improve the removal efficiency of foulants. Using sponge ball is another common method to

assist in scraping the deposits off the membrane surface. New studies for non-conventional physical cleaning may include ultrasonic and electrical cleaning. Ultrasonic cleaning prevents foulants adhering to the membrane surface by using high-frequency to agitate the feed. Electrical cleaning involves application of voltage across the membrane to pushing charged deposits away. Physical cleaning may prolong the membrane span since it is less likely to cause membrane degradation or damage compare to chemical cleaning. However, once severe fouling occurs, chemical cleaning is more effective removing the irreversibly attached foulants.

1.4.2 Chemical cleaning

Six categories of cleaning agents are commonly used in the industry: acid, alkalis, oxidizers, surfactants, chelating agents and enzymes[23,34–38]. Acids are primarily used to target inorganic fouling to dissolve precipitates of inorganic salts or oxide film. Weak acids, such as citric and oxalic acid, are favored over strong acids like hydrochloric, nitric, and sulfuric acid because it does not corrode the membrane surface. Sodium hydroxide and potassium hydroxide are widely used alkaline solution to promote hydrolysis of carboxylic and phenolic functional groups in proteins and polysaccharides into smaller amides and sugars. Alkalis also encourages neutralization of weakly acidic organic matters, expansion of natural organic matters and saponification of fats and oils leading to effective removal of foulants. Oxidizers including sodium hypochlorite and hydrogen peroxide are known to disinfect and oxidize organic and biological foulants. Anionic and non-ionic surfactants, such as sodium dodecyl sulfate and Tween 20, are generally recommended to help wet the surface of the fouled membrane and reduce the adhesion forces between the fouling layer and the membrane. Chelating agents can form strong complexes with multivalent metal ions such as calcium via ligand exchange reaction and disrupt the intermolecular bridges within the foulant as well as those between the foulant and the membrane surface. Enzymes

degrade organic molecules by targeting specific bonds. Despite their biodegradable and environmentally friendly features, enzyme cleaning is difficult to control since the activity of enzymes is limited by various environmental factors and specificity of the fouling layer.

1.5 Research Objectives and Dissertation Organization

While abiotic fouling including scaling, organic and colloidal fouling, can be manageable, biofouling, which accounts for more than 45% of all membrane fouling[39], is more complicated to control. Moreover, the cost associated with biofouling in membrane filtration for water treatment processes is between 25 to 50 % of the total operational cost[40] due to higher requirements of energy, maintenance of the system and periodic replacement of membranes. Therefore, my research focuses on biofilm removal.

Current biofilm management relies on using chemical cleaning agents jointly or sequentially to enhance the membrane cleaning efficiency but are not specifically tailored to the fundamental properties of the biofilms. Hence, cleaning strategies often rely on a trial-and-error approach, which is an iterative process to test various cleaning agents in the hope of removing the biofilm. This leads to an excessive and indiscriminate use of cleaning agents that causes an increased economic and environmental cost associated with the production and disposal of large quantities of these chemicals. Therefore, the overall objective of this project was to develop a scientifically-informed, sustainable biofilm management strategy by examining biofilm cleaning methods and studying the fundamental interactions between different elements of a microbial biofilm and representative cleaning methods.

It is hypothesized that interactions between specific cleaning methods and individual species composing the microbial biofilm will determine the effectiveness of the cleaning strategy. In addition, it is hypothesized that the biofilm composition affects the properties of the biofilm (i.e.

hydrophilicity) that will dictate the efficacy of the cleaning strategy. In order to test these hypotheses, the following objectives have been drawn. The first objective is to determine the impact of representative cleaning methods on individual biofilm components. The second objective is to determine the impact of representative cleaning methods on biofilm component mixtures and lastly, to optimize microbial management practices based on the outcomes from objectives 1 and 2. The outcome of these three objectives will give fundamental knowledge of the contribution of each of the biofilm components and an optimized strategy for cleaning the fouled UF membranes.

This dissertation comprises four chapters, which include detailed research descriptions to address the objectives stated above. Chapter 1 presents an introduction to membrane filtration along with its importance and advantages in water treatment processes and challenges that it faces to set the framework for the following chapters. Chapter 2 evaluates the flux recovery of fouled membranes with various chemical cleaning agents. An ultrafiltration membrane is fouled with either a single biofilm component, mixture of biofilm components that mimic bacterial biofilms or an actual bacterial biofilm component. Chapter 3 analyzes the efficacy of chemical cleaning agents to remove major biofilm components absorbed onto a quartz crystal microbalance sensor. The senor is coated with a polymer layer that simulates an ultrafiltration membrane surface. The result is compared to the outcome from the experiments conducted in Chapter 2. Lastly, Chapter 4 summarizes the results from Chapter 2 and 3 and concludes with suggestions for future avenues of research to extend this work.

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Chapter II.

Impact of Physical and Chemical Cleaning Agents on Specific Biofilm Components and the Implications for Membrane Biofouling Management

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2.1 Introduction

Membrane fouling remains a critical problem faced during membrane-based water treatment processes. Biofouling, in particular, accounts for more than 45 % of all membrane fouling events[1] and remains a complicated process to control. In biofouling, bacteria grow and multiply on the membrane surface, all the while exuding extracellular polymeric substances (EPS). It is this aggregate material including cells and exuded organics that form a biofilm.[2] The biofilm forms a barrier between the membrane surface and the feed solution, which increases mass transfer resistance and leads to a decline in flux and ion rejection, and membrane degradation.[3,4] While many strategies have been proposed to prevent biofilm formation on membrane surfaces, to date, no strategy has been successfully implemented at the commercial scale that completely prevents membrane biofouling. Thus, membrane operators rely on biofouling management strategies that limit biofilm formation and recover membrane performance. Generally, these strategies include the use of physical methods such as backwashing and turbulent mixing (mesh spacers and air scouring) that remove the foulants from the membrane surface,[5] and chemical methods that break down the chemical bonds holding the biofilm together and to the membrane surface itself.[6] Typically, a combination of both physical and chemical cleaning steps are needed to maintain optimum membrane performance.[7]

Extensive characterization studies have identified the different chemical constituents that compose microbial biofilms.[8–11] EPS, which account for over 90 % of the biofilm mass, is produced and released by microorganisms and forms a protective gel-like layer that encompasses the microbial cells and any waste products they generate.[12] Therefore, EPS is composed of the same molecular species that comprise the microorganisms themselves, including polysaccharides, proteins, deoxyribonucleic acid (DNA), and lipids.[13] However, while the same basic constituents

are found in all biofilms, the mass ratios between these constituents differs between biofilms formed by different species. One study that investigated the composition of a gram-negative *Pseudomonas aeruginosa* biofilms found extracellular polysaccharides and proteins made up approximately 60 % and 5 % of the total biofilm weight, respectively,[14] while in an *Escherichia coli* biofilm, another gram-negative bacterial, polysaccharides and proteins made up approximately 10 % and 55 % of the total biofilm weight, respectively.[15] Another study that investigated the composition of a gram-positive *Bacillus subtilis* biofilm, determined that the EPS was composed of 5 % polysaccharides and 60 % of proteins.[16] Although the composition of the EPS can vary greatly between biofilms, each component of the EPS are crucial to the survival of the bacterial biofilm, which contribute to surface adhesion, cell cohesion, aggregation, protection and water retention of the biofilm.[17] Specially, polysaccharides are known to be responsible for the foundation of the microbial microcolonies and mechanical stability of the biofilm.[12] Extracellular proteins, another major component of biofilms, are known to form weak bonds with EPS polysaccharides that hold the biofilm together.[18] In particular, lectins are sugar binding proteins that connect the cell wall to the polysaccharides.[19] Although extracellular DNA (eDNA), which is released by lysed microbial cells, has been considered a minor component of biofilms, recent studies indicate that it is in fact an integral part of the biofilm structure.[20] Lipids are another minor component of the biofilm matrix. However, the hydrophobic properties of the lipid are important for bacterial attachment and detachment to the surface.[21] Thus, understanding the interactions of individual elements comprising the EPS will give insight into the mechanisms involved in creating and removing biofilms on membrane surfaces.

Current biofilm management practices rely on a combination of physical and chemical cleaning methods designed to remove deposited biofilms.[5–7,22,23] Chemical cleaning agents

commonly used for membranes fall into six categories: acids, alkalis, oxidizers, surfactants, metal chelating agents and enzymes. Acids are used to target inorganic substances, such as calcium carbonate and metal oxides, that can be part of the organic biofilm matrix.[22] However, low pH can decrease the net charge of the organic, which enhances fouling and flux decline.[24] On the other hand, alkalis are used to remove organic substances by hydrolyzing or solubilizing them into smaller particles. For example, hydroxide ions promote cleavage of polysaccharides and proteins into smaller sugars and amino acids.[22] Oxidizers kill microorganisms and oxidize functional groups of organic foulants to ketonic, aldehydic, and carboxylic groups, which reduces the adhesion between foulants and membrane surface.[5] Surfactants form micelles around hydrophobic organic molecules to solubilize and remove them from the membrane surface. Because of their low surface tension, membrane surface wettability increases and promotes other cleaning agents to penetrate into the biofilm.[23,25] However, the use of surfactants in the confined spaces of a membrane module is problematic due to foaming and the difficulty of rinsing residual surfactant after the cleaning process.[26] Metal chelating agents replace metal ions, especially divalent cations, by ligand exchange reactions, which removes the intermolecular bridges within the EPS matrix as well as those between the biofilm and the membrane surface.[5] It was demonstrated that in the presence of calcium ions the cross-linked, gel-layer structure of alginate, commonly used to simulate EPS polysaccharide, was destroyed by a metal chelating agent, ethylenediamine tetraacetic acid (EDTA).[27] Enzymes are excellent at targeting specific bonds in the EPS structure and their hydrolytic power is much greater than acids or alkalis, which makes them effective cleaning agents.[23] Because of their high hydrolyzing power and biodegradability, studies on the use of enzymatic solutions has expanded over the past years.[28–31] However, enzyme activity is

limited to specific organic matter at certain pH and temperature levels, and enzymes are difficult to recover when solubilized in aqueous medium.[1]

Although most standard biofilm removal treatments involve using chemical cleaning agents jointly or sequentially,[32,33] chemical cleaning regimes recommended by membrane manufacturers do not yield consistent results due to a lack of understanding of the relationship between specific biofilm composition and cleaning agents. To develop a feasible and optimal cleaning protocol, extensive studies have investigated the impact of various cleaning agents on biofilm removal from membrane surfaces. Some studies focused on the efficacy of different cleaning methods on specific biofilm components such as alginate[31,34–37] or bovine serum albumin (BSA)[28,38–41] to represent major components of the EPS. However, these single-component studies are insufficient to describe the chemical cleaning process of a complex biofilm matrix, which is composed of a mixture of organic molecule types. Further studies on cleaning mixtures of these biofilm components have been done[32,34,42] yet they do not resemble an actual bacteria biofilm but rather a random mixture of biofilm components. Numerous studies have investigated the impact of various cleaning agents on membranes fouled during surface water treatment[33,43–45] or waste water treatment;[25,46–48] these studies suggest different cleaning agents or processes. For membranes fouled by surface water, Liikanen et al. (2002) found alkaline cleaners with metal chelating agents to be the most efficient cleaning method,[33] while Zondervan et al. (2007) found alkaline and oxidizing cleaning agents to give the best overall cleaning results.[45] Madaeni et al. (2010) showed that alkaline and detergent cleaning followed by acid provided effective recovery for waste water fouled membranes,[25] while Ang et al. (2011) highlighted that the addition of NaOH with other chemical agents can enhance the overall cleaning performance, where the addition of NaOH increased the cleaning efficiency to 94 %.[46]

In this study, the interactions between a range of cleaning agents (alkalis, oxidizers, surfactants, metal chelating agents), electrolyte solutions, and individual biofilm components (polysaccharides, proteins, DNA, lipids) are evaluated with the goal of identifying the cleaning efficacy of each cleaning agent towards individual biofilm components. Since no inorganic species were used in these experiments, acid cleaning was not evaluated. In addition to chemical cleaning, the role of hydrodynamic cleaning (cross-flow washing and backwashing) in removing specific biofilm components is identified. Furthermore, the efficacy of cleaning agents towards model EPS mixtures designed to mimic gram-negative and gram-positive bacterial biofilms were tested and compared to an actual gram-negative EPS biofilm. The goal of this work is to inform researchers and membrane operators about the importance of identifying the specific composition of a biofilm, and the tailoring of cleaning strategies that generate consistent membrane recoveries.

2.2 Experimental Section

2.2.1 Organic foulants and P. aeruginosa EPS

The organic foulants chosen to represent the polysaccharides, proteins, DNA and lipids found in biofilms were alginic acid sodium salt from brown algae (Sigma-Aldrich, St. Louis, MO), BSA (Sigma-Aldrich, St. Louis, MO), DNA sodium salt from salmon testes (Sigma-Aldrich, St. Louis, MO), and octanoic acids (ULTRA Scientific, N. Kingstown, RI), respectively. The feed solution was either composed of a single component foulant or a mixture of foulants that represented bacterial EPS (see Table 2.1), with the total mass of the foulants used to foul the membrane fixed to 5 mg. In these mixtures, the composition of DNA includes the dry weight

percent of ribonucleic acid, as well. Foulants were well mixed and freshly prepared in two electrolyte solutions: 10 mM NaCl or 8.5 mM NaCl with 0.5 mM $CaCl_2$, filtered with a 0.22 µm Millipore PVDF filter. This ionic strength was chosen to represent the lower limit of groundwater and the upper limit of surface water that covers most of drinking water sources.[50,51] As the presence of divalent cations is known to enhance biofouling,[52] the amount of biofilm attaching and detaching to the membrane surface in the absence or presence of Ca^{2+} ions will indicate the role of divalent cations in these processes.

Table 2.1 Compositions of bacterial EPS.

Bacteria type	Polysaccharide	Protein	DNA	Lipid	Ref
gram – (*P. aeruginosa*)	60 %	5 %	20 %	15 %	[35,70]
gram + (*B. subtilis*)	5 %	60 %	20 %	15 %	[37]

To compare the results from the model EPS mixtures, an actual EPS was used in the fouling/cleaning process. EPS extracted from a *P. aeruginosa* PAO1 biofilm[53] was used to foul the UF membrane and was subsequently cleaned. In these experiments, 2.5 mg of EPS was used in each experiment and the EPS was mixed in 8.5 mM NaCl with 0.5 mM $CaCl_2$ electrolyte solution to ensure maximum fouling.

2.2.2 Chemical cleaning agents

Among the six commonly used chemical cleaning agents for biofilm management, enzyme and acid were eliminated in this study. As previously mentioned, despite its effectiveness to remove inorganic precipitates, acid enhances fouling by neutralizing or changing the net charge of the organic matter, making it a poor cleaning agent for organic foulant removal compared to alkalis. In addition, single enzymes are not suitable to target bacterial biofilms, which consist of a mixture of macromolecules. Therefore, the efficacy of the other four types of agents (an alkali, oxidizer, surfactant, and metal chelating agent) were tested. The chemical cleaning agents included NaOH

(pH 12) as an alkali, 100 ppm NaOCl (oxidizer), 10 mM Dodecyl sulfate sodium salt (SDS) (surfactant), and 5 mM ethylene diamine tetra acetic acid (EDTA) (chelator) at pH 11 adjusted by NaOH. Each cleaning agent was prepared using Millipore water without further purification.

2.2.3 UF membrane and flow cell

A commercially available PE-10 polyethersulfone membrane (Nanostone Water, Carlsbad, CA) with a molecular cut-off (MWCO) of 10 kDa was used. The membrane cell unit was designed using SolidWorks CAD Software and constructed using a Hatchbox Alpha 3D printer using acrylonitrile butadiene styrene filaments that allowed for the construction of a complex, low cost, reproducible flow cell. The dimensions of the 3D-printed flow channel were 7.3 cm × 3.3 cm, with a channel height of 0.36 cm. To prevent shear stress from damaging the fouled layer, the inlet cross-flow was controlled by a syringe pump at an average flow velocity of 0.056 cm/s (corresponding to a shear rate of 0.94 s^{-1}) with a transmembrane pressure of 6 psi.

2.2.4 Fouling and chemical cleaning protocol

Membranes were stabilized and equilibrated with Millipore water for 15 minutes at constant pressure (6 psi). Initial flux was measured using foulant-free electrolyte solution, typically ranging from 40-50 LMH. A fixed mass of foulants were deposited onto the membrane operating in a dead-end filtration mode. The permeate was not recycled. Next, the fouled membrane was exposed to various cleaning solutions in two steps. First, the membranes were operated in a cross-flow configuration with the cleaning solution flowing across the membrane surface for 15 minutes in the absence of any imposed transmembrane pressure. Following the cross-flow cleaning step, the membranes were backwashed with the cleaning solution for 10 minutes at 1 psi and a crossflow velocity 8 times lower than during cross-flow cleaning. As a control, fouled membranes were also either exposed to only cross-flow cleaning, or backwash cleaning with the foulant-free electrolyte

solution. To verify the effect of the chemical cleaning agents on the membrane itself, pristine membranes were cleaned with each chemical cleaning agents, using the same cleaning protocol used for the fouled membranes. Fouling and cleaning experiments were each performed in triplicates and 95 % confidence intervals were calculated to gauge the effectiveness of the cleaning process.

2.2.5 Membrane surface morphology

Scanning electron microscopy (SEM) (FEI NovaNanoSEM 450) and atomic force microscopy (AFM) (MFP-3D, Asylum Research) were used to explore the surface morphology of the membrane. SEM images of the pristine membrane, fouled membranes with individual foulants (alginate, BSA, DNA) in 10mM NaCl and NaOH cross-flow cleaned membrane followed by backwash cleaning were taken. AFM images of the pristine membrane, alginate and BSA fouled membrane were taken and the surface roughness of each membrane was measured. AFM was used in tapping mode in water with silicon nitride probes (NCHV-A, Bruker).

2.3 Results and Discussion

2.3.1 Cleaning membranes fouled by individual foulants

In this part of the investigation, PES UF membranes were fouled with 5 mg of individual fouling components (alginate, BSA, or DNA) in a dead-end membrane configuration. Fouling the membrane with 5 mg of octanoic acid did not result in any appreciable flux decline (data not shown), likely because the foulant was significantly smaller than the membrane's MWCO and no significant adsorption occurred between the foulant and the membrane. Thus, only the three larger foulants were considered in this stage of the study. For the initial foulant deposition, the fouling components were suspended in either 10 mM of NaCl or 8.5 mM NaCl and 0.5 mM $CaCl_2$. In all cases, the foulant molecule was larger than the membrane's MWCO value (10 kDa) with 100 %

rejection. The resulting flux declines were significantly different (Figure 2.1, gray bars) even though the same mass of foulants was deposited on the membrane. Specifically, membranes fouled by alginate with 10 mM NaCl experienced the greatest flux decline (75.9 ± 1.23 %), while alginate in the presence of Ca^{2+} exhibited slightly less flux decline (71.3 ± 2.23 %) (Figure 2.1a, b). BSA-fouled membranes experienced a flux decline of only 38.0 ± 1.51 %, while DNA-fouled membranes experienced a flux decline of 59.4 ± 1.14 %. Flux declines induced by BSA and DNA were not sensitive to Ca^{2+} (Figure 2.1). These results are in line with previous investigations, which observed the three macromolecules to behave differently as membrane foulants due to their different structures.[12] In particular, depending upon the presence of Ca^{2+}, alginate forms either a globular structure or a dense gel-like structure on the membrane surface.[36,54] The globular protein BSA forms a relatively loose layer on the membrane surface and does not restrict flux as much as a continuous gel-layer, which leads to a relatively lower flux for the same mass.[55,56] DNA, which is typically a long, relatively linear molecule when not associated with proteins, produces a fouling layer that falls in between BSA and alginate.[57] SEM images of the fouled membrane reveal a uniformly covered surface, although some cracks in the alginate layer are evident (Figure 2A.1). We speculate that these cracks are a result of the drying process used to image the membranes. Meanwhile, the pristine membrane surface is completely clear of any obvious deposited layer. To further compare the surface roughness of the pristine membrane and fouled membrane surface, AFM images were taken. However, no significant difference in the surface roughness was observed, with all membranes exhibiting an average roughness below 2 nm (Figure 2A.2).

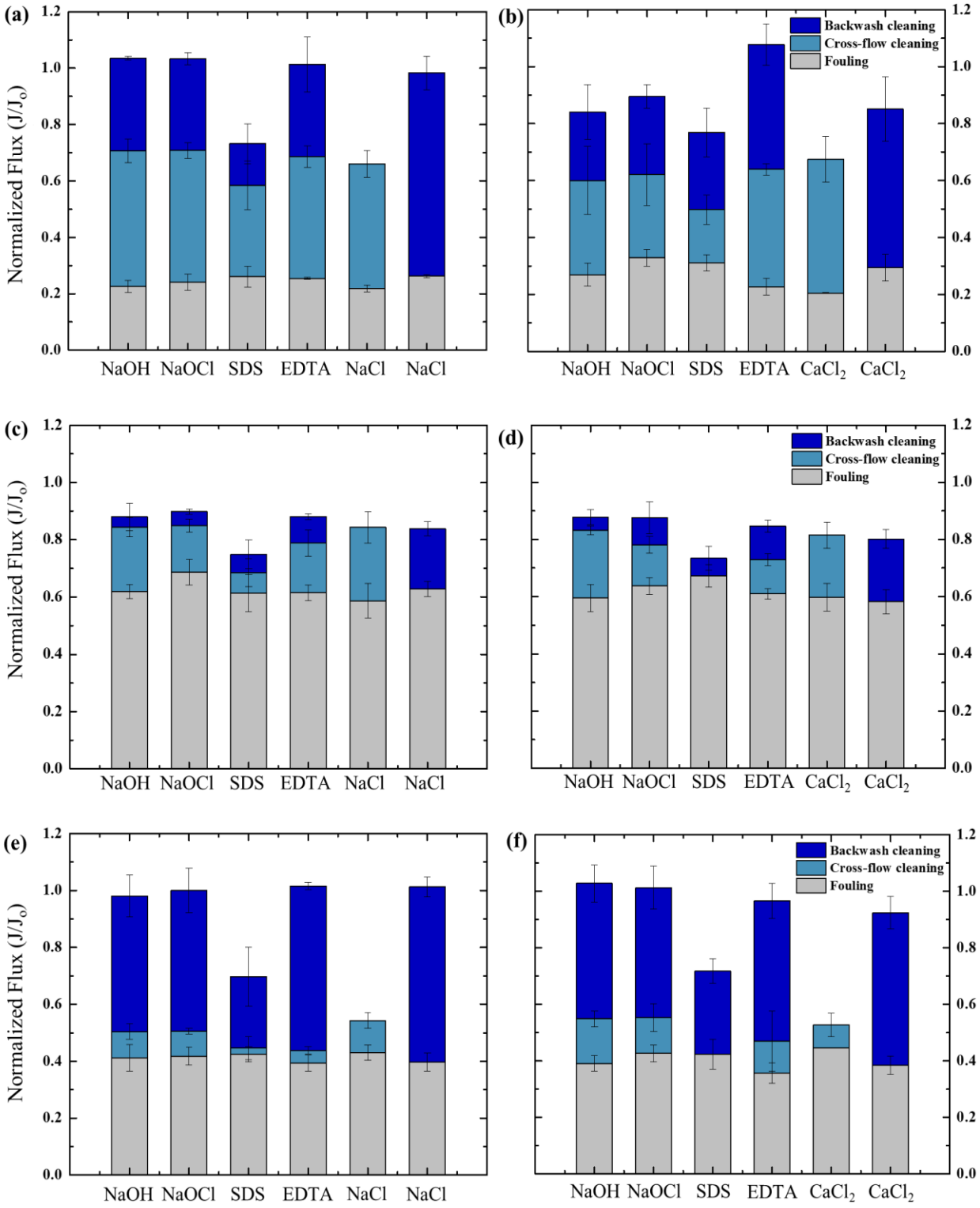

Figure 2.4 Normalized flux of membranes fouled (grey bars) by alginate (a,b), BSA (c,d) and DNA (e,f) and cleaned with various cleaning agents through crossflow (light blue bars) and backwash (dark blue bars); in Figures 2.1a,c,e the background electrolyte is 10 mM NaCl, while in Figures 2.1b,d,f the background electrolyte is 8.5 mM NaCl and 0.5 mM $CaCl_2$.

Once fouled, the membranes were exposed to 15 minutes of cross-flow (Figure 2.1, teal bars) followed by 10 minutes backwashing with a cleaning solution (NaOH, NaOCl, SDS, or EDTA) (Figure 2.1, blue bars). Additionally, the fouled membranes were either exposed to only cross-flow cleaning or only backwashing with the electrolyte solution (Figure 2.1). When pristine membranes were cleaned with NaOH, NaOCl, and EDTA no flux change was observed. However, when SDS was used, 25 % drop in flux was observed. We speculate that this is a result of incomplete removal of SDS from the system, despite the same rinsing duration that was applied to each cleaning agent. The addition of chemical cleaning agents, except SDS, to the cross-flow or backwash solutions did not significantly improve the cleaning of alginate-fouled membranes in the presence 10 mM NaCl over cleaning with only the electrolyte (Figure 2.1a). Cleaning with SDS resulted in worse cleaning performance, likely due to the large amount of foaming during the introduction of SDS, which prevented accurate flux measurements. SEM images of the cleaned membrane show a surface that looks far "cleaner" than the alginate-fouled one, although some cracking is still evident, which indicates that alginate is still there, even though complete flux recovery was achieved (Figure 2A.1b). Thus, it is possible that the cleaning process loosened the fouling layer, which allowed for flux recovery, but did not completely remove the layer, which could serve as a conditioning layer in future rounds of filtration. In the presence of 8.5 mM NaCl and 0.5 mM $CaCl_2$ (Figure 2.1b), cross-flow cleaning was as effective as in the presence of 10 mM NaCl, regardless of the cleaning chemicals used. However, backwashing the membrane was not as effective and did not result in complete flux recovery, except when EDTA was added. Calcium ions are known to act as a "bridge" between neighboring alginate molecules as well as between alginate and the negatively charged membrane surface.[17] Although the PES membrane material does not have ionizable functional groups, the surface will acquire a negative charge due to the

presence of anions, which adsorb to the membrane surface because they are less hydrated than cations, and as a result can approach more closely to nonpolar or hydrophobic surface of the membrane.[58,59] One study observed calcium ions to induce a stronger interaction between a particle representing a foulant with dominantly carboxylic functional groups (similar to alginate) and the negatively charged membrane, while no adhesion force was measured when only monovalent ions were present in the solution.[27] Thus, we speculate that the backwashing step was not able to remove these "bridged" molecules even when chemicals like NaOH and NaOCl that can "break" the macromolecular bonds were added. However, the addition of EDTA, which chelates Ca^{2+} broke the "bridge" and resulted in complete alginate removal and flux recovery.

Cross-flow cleaning of BSA-fouled membranes only had limited effectiveness in cleaning the membrane surface, regardless of what cleaning agent was used (Figure 2.1c,d). The average normalized flux of the BSA-fouled membrane was greater than 60 %, yet cross-flow cleaning recovered flux only up to 84 % of its initial value. In contrast, the flux of alginate-fouled membranes recovered by up to 48 % from the cross-flow cleaning. SEM images of the cleaned membrane show a surface that is still covered by the foulant, as evident by the extensive cracking (Figure 2A.1d). Except for SDS, the electrolyte solutions were as effective as other chemical solutions to remove BSA and were not influenced by the presence of Ca^{2+}. Critically, the use of EDTA did not improve flux recovery in the presence of Ca^{2+}, illustrating the difference between alginate and BSA fouling. Clearly, Ca^{2+} does not interact with BSA in the same way as with alginate and does not facilitate the formation of "bridges" with other BSA molecules or with the membrane. Backwashing BSA-fouled membranes was not as effective at recovering flux compared to alginate. While backwashing with the chemical cleaning solution after cross-flow cleaning led to about 30 % increase in flux recovery for the alginate-fouled membrane, flux

recovery for BSA-fouled membrane only increased 5 - 10 %. Except for NaOCl in the presence of 10 mM NaCl and NaOH in the presence of 8.5 mM NaCl and 0.5 mM $CaCl_2$, the electrolyte solutions were as effective as the chemical cleaning solutions and showed no significant difference between cross-flow cleaning or backwashing. This further illustrates the difference between BSA and alginate fouling, where BSA molecules are able to more tightly attach to the membrane surface and resist removal by hydrodynamic forces, resulting in incomplete flux recovery. This is likely a result of the hydrophobic interactions between the BSA and the surface, which causes a significant breakdown in the secondary structure of the BSA. BSA is known to interact with hydrophilic surfaces through polar carboxyl groups. However, on hydrophobic surfaces, methyl groups from the BSA molecule can interact with the phenyl groups of the membrane surface, which results in a loss of α-helical domains that and allow more molecules to adsorb as compared to the number on a hydrophilic surface.[60] As observed in the alginate case, cleaning with the surfactant yielded poor results that are likely caused by the excessive foaming in the system.

Cross-flow cleaning with the cleaning solutions or electrolyte solutions had little effect on recovering the flux of the DNA-fouled membrane, whether in the absence or presence of Ca^{2+}. (Figure 2.1e,f). However, significant flux recovery was observed with backwashing. Backwashing with the cleaning solutions and both electrolyte solutions, with the exception of SDS, resulted in near complete flux recovery. SEM images of the membranes after cleaning show a surface with extensive cracking, indicating that while flux was fully recovered, significant organic material remained on the membrane surface, which could serve as a conditioning layer in later rounds of filtration (Figure 2A.1f). This behavior can be potentially explained by the type of fouling layer generated when DNA is deposited on the membrane surface. Previous studies observed significant flux decline during plasmid DNA filtration due to membrane pore blocking and showed that the

flux could be restored by periodic back-pulsing to push these plasmids out of the pores.[61] This supports our findings that backwashing is highly effective at recovering the membrane flux and it can be inferred that the DNA molecules do not form strong interactions with the membrane surface. Similar to BSA-fouled membranes, the efficiency of the short cross-flow cleaning and the backwashing with the cleaning solutions and electrolyte solutions showed no significant difference between membranes fouled with 10 mM NaCl or 8.5 mM NaCl and 0.5 mM $CaCl_2$, indicating that Ca^{2+} is not effective at bridging DNA molecules.

Overall, the cleaning efficiency of the electrolyte solutions was observed to be comparable to the cleaning solutions when membranes fouled by individual biofilm components were cleaned for a short amount of time (15 min). The notable exception was when cleaning alginate-fouled membranes in the presence of Ca^{2+}, where cleaning with EDTA yielded more effective cleaning performance. Previous investigations observed that cross-flow cleaning with various concentration of NaCl on organic fouled membranes in the presence of Ca^{2+} were quite effective.[35,46] Lee et al. (2007) showed that the cleaning efficiency of NaCl was around 55 % and 90 % at concentration of 10 mM and 100 mM, respectively, on an alginate-only fouled reverse osmosis membrane in the presence of Ca^{2+}. However, other studies showed that the cleaning efficiency of backwashing with 0.5 mM $CaCl_2$ was low.[37,62] We speculate that the presence of Na^+ in the backwash used in our study, which is roughly 20-fold higher than the concentration of Ca^{2+}, could replace the Ca^{2+} bonds inside the fouled layer. It is hypothesized that exposing the electrolyte solution to the organic fouled layer will swell and lessen its structural integrity. As a result, ion-exchange between Na^+ and Ca^{2+} can take place, ultimately releasing the Ca^{2+} and breaking down the fouled layer.[35]

2.3.2 Cleaning membranes fouled by mixtures of foulants

To test the effectiveness of the cleaning methods on biofilm-fouled membranes, synthetic mixtures of biofilm components that represent gram-negative (*Pseudomonas aeruginosa*) and gram-positive (*Basillus subtilis*) bacterial EPS were used to foul a membrane in a dead-end configuration.[63,64] The main difference between the two mixtures is the ratio between the amount of polysaccharides and proteins.[1-3] Gram-negative bacteria typically have a higher polysaccharide content (alginate-dominant), while gram-positive bacteria have a higher protein content, although exceptions do occur.[65,66] Both synthetic EPS mixtures had the same amount of DNA and fatty acids (Table 1). Several studies reported the influence of fatty acids on membrane fouling.[67,68] Despite the fact that octanoic acid, a hydrophobic, saturated fatty acid, is significantly smaller than the pores of a typical ultrafiltration membrane, it can cause drastic flux decline depending on the octanoic acid concentration and the pH of the solution.[69] In this study, during the individual component fouling, the amount of octanoic acid (5 mg) was insufficient to cause flux reduction. However, previous work has demonstrated that fatty acids can enhance calcium binding to proteins[70] and alter the molecular interactions between other organic compounds and surfaces even at very low concentrations.[71] Thus, it was expected that the addition of octanoic acid to the EPS mixture would complicate the cleaning process of membranes fouled by the complex mixture representing bacterial EPS.

2.3.2.1 Cleaning gram-negative EPS (alginate-dominant mixture) fouled membranes

A mixture of alginate, BSA, DNA and octanoic acid (total 5mg; Table 2.1) was used to foul the membrane in the presence of either 10 mM of NaCl or 8.5 mM NaCl and 0.5 mM $CaCl_2$ as the background electrolyte. Once the membrane was fouled, cross-flow and backwash cleaning with NaOH, NaOCl, SDS, EDTA or electrolyte solution was performed (Figure 2.2). The flux

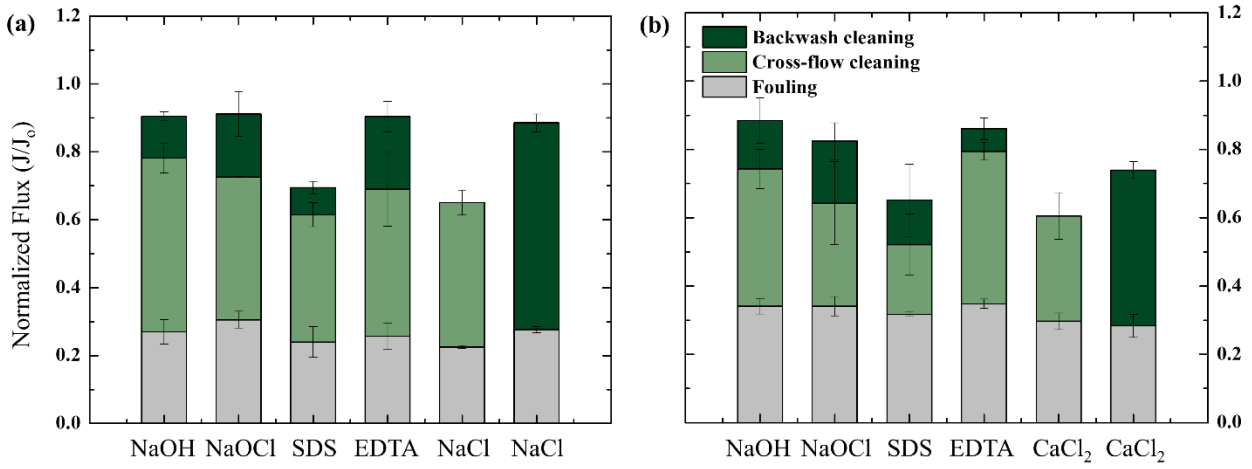

Figure 2.5 Normalized flux of foulant mixture representing gram negative bacteria EPS composition (alginate:BSA:DNA:octanoic acid = 60:5:20:15) with (a) 10 mM NaCl (b) 8.5 mM NaCl and 0.5 mM $CaCl_2$ cleaned by various solutions.

decline induced by the alginate-dominant mixture was greater in the presence of 10 mM NaCl than in the presence of 0.5 mM Ca^{2+} (Figure 2.2a,b, gray bars). In addition, flux decline by the EPS mixture was lower than when alginate alone was used to foul the membrane (Figure 2.1a,b). The flux decline with the EPS mixture was 73.1 ± 1.54 % and 67.5 ± 1.48 %, while alginate-only fouled membrane resulted in 75.9 ± 1.23 % and 71.3 ± 2.23 % for the 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively. Other studies showed that the presence of protein in the alginate mixture slightly loosens the cake layer by disrupting the binding between alginate aggregates. [56,72] The addition of other components in the alginate-dominant mixture, DNA and octanoic acid, could also explain the different flux decline behavior.

Unlike the alginate-only fouled membrane, where no significant difference was observed between the cleaning solutions and 10 mM NaCl (except SDS), cross-flow cleaning of alginate-dominant EPS fouled membranes with NaOH was slightly more effective than 10 mM NaCl and SDS (Table 2.2). However, the ability of each cleaning solution to remove the alginate-only foulant or alginate-dominant mixture was not statistically different. In the presence of 8.5 mM NaCl and 0.5 mM $CaCl_2$, NaOH and EDTA yielded more effective flux recovery than the electrolyte solution

alone. When the membrane was fouled in the presence of Ca^{2+}, EDTA was 15.5 % more effective in cleaning the alginate-dominant EPS fouled membrane than the alginate-only fouled membrane. It is likely that the lower mass of alginate in the EPS mixture (compared to the pure alginate) made EDTA cleaning more effective, since it specifically targets the Ca^{2+} – alginate structure. This is in contrast to NaOH and NaOCl, which indiscriminately attack different chemical bonds and modify the foulant-membrane interaction.

Table 2.2 Summary of normalized flux of alginate-dominant EPS fouled membranes and alginate-only fouled membranes after cleaning with various cleaning agents.

	Cross-flow cleaning				Backwashing			
Background solution / Cleaning agents	10 mM NaCl		8.5 mM NaCl & 0.5 mM $CaCl_2$		10 mM NaCl		8.5 mM NaCl & 0.5 mM $CaCl_2$	
	EPS mixture	Single foulant	EPS mixture	Single foulant	EPS mixture	Single foulant	EPS mixture	Single foulant
NaOH	78.2 ± 4.40	70.7 ± 4.22	74.3 ± 5.70	60.0 ± 12.0	90.6 ± 1.23	103 ± 0.70	88.5 ± 6.64	84.0 ± 9.69
NaOCl	72.6 ± 0.20	70.8 ± 2.78	64.3 ± 12.2	62.1 ± 10.8	91.1 ± 6.63	103 ± 2.19	82.4 ± 5.42	89.6 ± 4.11
SDS	61.5 ± 3.50	58.4 ± 8.62	52.2 ± 9.01	49.8 ± 5.05	69.4 ± 1.92	73.2 ± 7.00	65.2 ± 10.5	76.9 ± 8.57
EDTA	69.0 ± 10.9	68.6 ± 3.81	79.5 ± 2.67	64.0 ± 2.03	90.4 ± 4.49	101 ± 9.73	86.1 ± 3.13	108 ± 7.24
10 mM NaCl	65.1 ± 3.68	66.1 ± 4.73	—	—	88.5 ± 2.65	98.3 ± 5.89	—	—
8.5 mM NaCl & 0.5 mM $CaCl_2$	—	—	60.5 ± 6.83	67.5 ± 7.98	—	—	73.9 ± 2.47	85.1 ± 11.3

In contrast to the complete flux recovery observed when an alginate-only fouled membrane was backwashed in the presence of 10 mM NaCl (Figure 2.1a), backwashing the membrane fouled by the alginate-dominant mixture did not lead to complete flux recovery, regardless of the cleaning chemicals used (Figure 2.2a, dark green bars, Table 2.2). The addition of NaOH and NaOCl to the cleaning solution during cross-flow cleaning did achieve a somewhat higher degree of flux recovery compared to cross-flow cleaning with only NaCl (Figure 2.2a, light green bars, Table

2.2). This trend was also observed when the membrane was fouled in the presence of 0.5 mM Ca^{2+}, although in this case, cleaning with EDTA resulted in the highest degree of flux recovery (though not to full flux recovery; Figure 2.2b, Table 2.2). The difference between cleaning alginate-only and alginate-dominant EPS mixture could be explained by the contribution of other foulants in the feed solution. Other foulants mixed with alginate can form specific bonds with both the alginate and the membrane surface, which can better withstand both chemical and hydrodynamic cleaning. The presence of BSA in the EPS mixture, which can participate in stronger hydrophobic interactions with the membrane surface, may be particularly responsible for the incomplete flux recovery observed during the backwashing of the EPS mixture.

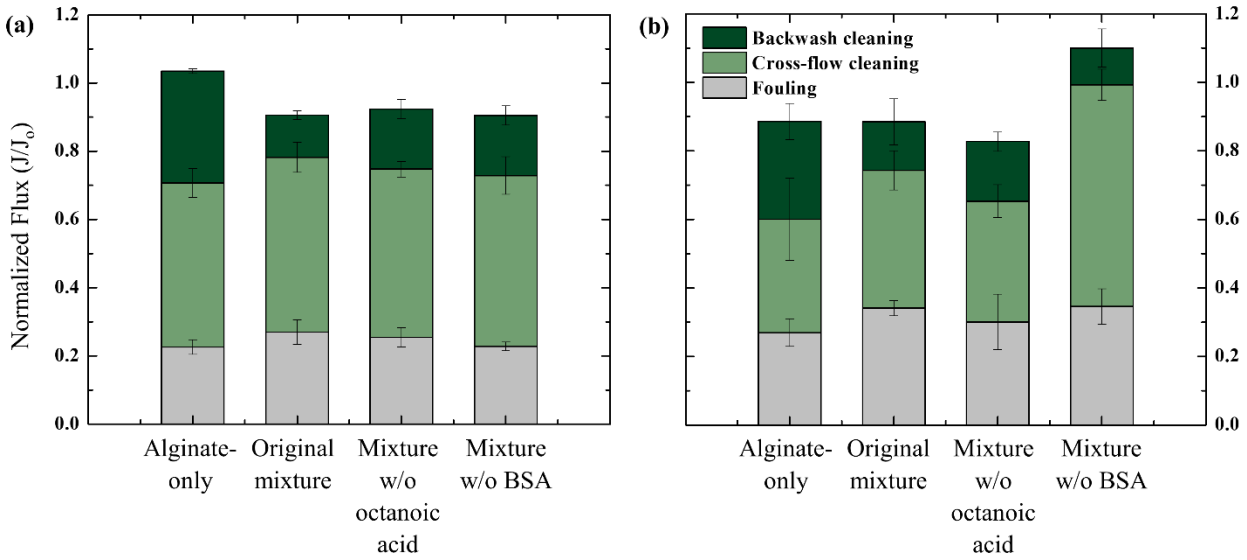

Figure 2.6 Normalized flux of alginate-only fouled membrane and alginate-dominant mixture fouled membranes with (a) 10 mM NaCl (b) 8.5 mM NaCl and 0.5 mM $CaCl_2$ cleaned by NaOH.

To evaluate the contribution of individual EPS components in the alginate-dominant EPS mixture to membrane fouling, mixtures that lacked one component from the original alginate-dominant mixture (the ratio of the other components was kept equal) were used to foul the membrane. In the previous section, we demonstrated that both alginate and DNA fouled

membranes can be fully recovered when backwashed. Therefore, we focused on the impact of BSA and octanoic acid on cleaning alginate-dominant mixtures. In Figure 2.3, we compare the cleaning results of membranes fouled with alginate-only, an alginate-dominant mixture representing EPS from gram-negative bacteria, a mixture without octanoic acid (alginate:BSA:DNA = 65:10:25), and a mixture without BSA (alginate:DNA:octanoic acid = 61.67:21.67:16.66). Based on our previous observations, cleaning with NaOH yielded the best results. However, in some cases, NaOCl and EDTA produced comparable results to NaOH. Specifically, EDTA showed better cleaning efficiency only when calcium ions were present. Thus, to clean the BSA-dominant fouled membrane, we focused our efforts on NaOH, which showed the best results for BSA removal using cross-flow cleaning and backwashing. The flux decline induced by alginate-dominant mixture without octanoic acid was 74.6 ± 2.81 % and 70.0 ± 8.12 %, while for alginate-dominant mixture without BSA the flux decline was 77.2 ± 1.27 % and 65.5 ± 5.16 % for the 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively (Figure 2.3a, b).

In the presence of 10 mM NaCl, cross-flow cleaning with NaOH (Figure 2.3a) recovered flux to similar values regardless of the different foulant composition, being 70.7 ± 4.22 %, 78.2 ± 4.40 %, 74.8 ± 2.32 % and 72.9 ± 5.51 % for pure alginate, alginate-dominant EPS mixture, alginate-dominant mixture without octanoic acid, and alginate-dominant mixture without BSA. However, only the membrane fouled with pure alginate fully recovered by backwashing with NaOH (Figure 2.3a). In the presence of 0.5 mM Ca^{2+}, removing octanoic acid and BSA from the EPS mixture had opposite effects (Figure 2.3b). When octanoic acid was removed, cross-flow cleaning was less effective at restoring flux compared to original EPS mixture. While the recovered flux of the original EPS mixture was 74.3 ± 5.70 %, the recovered flux of the alginate-dominant mixture without the octanoic acid was 65.3 ± 4.78 %. In contrast, when BSA was removed from

the mixture, cross-flow flushing was highly effective at restoring flux (99.3 ± 4.55 %). The relative concentration of BSA increased in the mixture as the octanoic acid was removed. Thus, we speculate that it is the presence of BSA in the EPS mixture that determines the effectiveness of the cleaning process, where its presence leads to less effective EPS removal. Another interesting observation is that in the presence of 0.5 mM Ca^{2+}, when BSA was absent from the mixture, membrane flux could be completely restored, which is different than the alginate-only case (Figure 2.3b). Thus, it is possible that octanoic acid counteracts the enhanced "stickiness" brought on by Ca^{2+} ability to bridge the alginate molecules, perhaps by hindering the ability of Ca^{2+} to attach to active groups on the membrane surface, for example, by sequestering Ca^{2+}. This finding is comparable with the results previously published where an increase in cleaning efficiency of NaOH (pH 11) was observed when the amount of BSA, Suwanee River natural organic matter and octanoic acid in the synthetic EPS mixture decreased while the amount of alginate increased.[46]

2.3.2.2 Cleaning gram-positive EPS (BSA-dominant mixture) fouled membranes

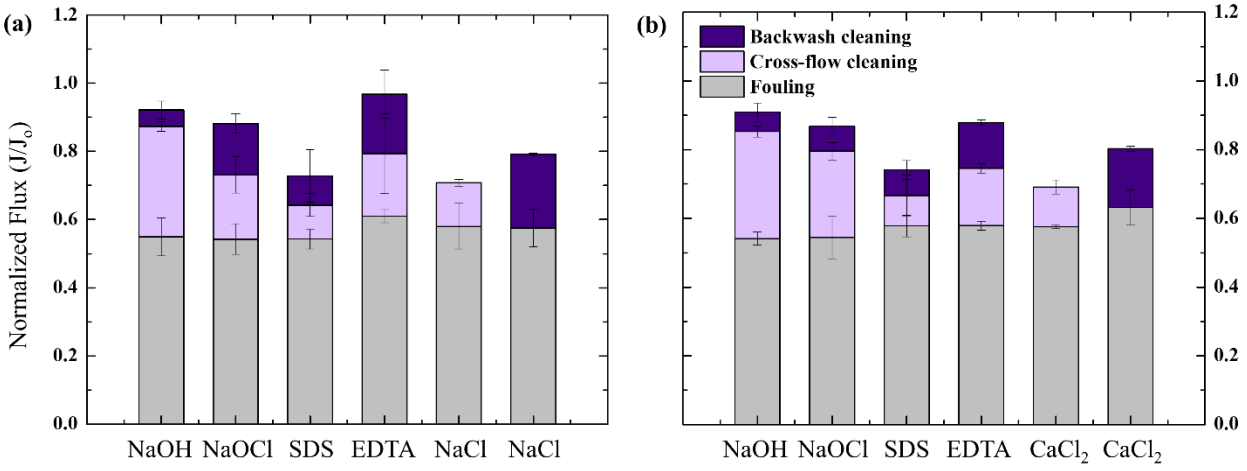

Figure 2.7 Normalized flux of foulant mixture representing gram positive bacteria EPS composition (alginate:BSA:DNA:octanoic acid = 5:60:20:15) with (a) 10 mM NaCl (b) 8.5 mM NaCl and 0.5mM $CaCl_2$ cleaned by various cleaning agents.

A mixture that represents a gram-positive (*Basillus subtilis)* bacterial EPS (prepared by mixing alginate, BSA, DNA and octanoic acid with a mass ratio of 5:60:20:15), was used to foul

the membrane surface, where the total amount deposited was 5 mg. The background electrolyte in the fouling step was either 10 mM NaCl or 8.5 mM NaCl and 0.5 mM $CaCl_2$. Once fouled, the membranes were cleaned by cross-flow and backwash using either NaOH, NaOCl, SDS, EDTA or the electrolyte solutions (Figure 2.4). As expected, the final flux of the membrane fouled by the BSA-dominant mixture was not influenced by the presence of Ca^{2+} (Figure 2.4a,b, gray bars), similar to the BSA-only conditions. As observed during alginate-dominant mixture fouling experiments, the flux of the multi-component BSA-dominant mixture fouled membrane was higher than the BSA-only fouled membrane. The flux decline of the membrane fouled by the BSA-dominant mixture was determined to be 43.8 ± 2.17 % and 43.3 ± 2.16 % of the original flux for the 10 mM NaCl and 8.5 mM NaCl and 0.5 mM $CaCl_2$ conditions, respectively. By comparison, the flux decline of the BSA-only fouled membrane was 37.2 ± 2.21 % and 38.9 ± 2.04 % for the 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively (Figure 2.1c, d). This again demonstrates the impact of the multiple components on the structure of the fouling layer, which impacts the ability of water to effectively pass through the fouling layer.

Similar to the alginate-dominant mixture fouled membrane, cross-flow cleaning of BSA-dominant mixture fouled membrane with NaOH was more effective than other cleaning solutions, including 10 mM NaCl. Cross-flow cleaning the BSA-dominant mixture was less effective with NaOCl and 10 mM NaCl than BSA-only foulant (Table 2.3). Cross-flow cleaning with NaOCl led to flux recovery of 73.2 ± 5.37 % and 84.9 ± 2.32 % for BSA-dominant mixture and BSA-only foulant, whereas, cleaning with 10 mM NaCl led to flux recovery of 70.8 ± 0.99 % and 84.4 ± 5.51 % for BSA-dominant mixture and BSA-only foulant. Cleaning with NaOH yielded similar cleaning performance of the BSA-dominant mixture and pure BSA, 87.3 ± 1.44 % and 84.4 ± 3.33 %, respectively. In the presence of 8.5 mM NaCl and 0.5 mM $CaCl_2$, NaOH and NaOCl

resulted in better flux recovery, each 85.3 ± 1.68 % and 79.5 ± 2.49 %, than the electrolyte solution (69.2 ± 2.07 %). However, the efficiency of each cleaning solutions to remove BSA-only foulant or BSA-dominant mixture were not statistically different (Table 2.3). Again, the use of EDTA did not improve the flux recovery as seen in the BSA-only fouled membrane in the presence of Ca^{2+}.

Table 2.3 Summary of normalized flux of BSA-dominant EPS fouled membrane and BSA-only fouled membrane after cleaning with various cleaning agents.

	Cross-flow cleaning				Backwashing			
Background solution / Cleaning agents	10 mM NaCl		8.5 mM NaCl & 0.5 mM $CaCl_2$		10 mM NaCl		8.5 mM NaCl & 0.5 mM $CaCl_2$	
	EPS mixture	Single foulant	EPS mixture	Single foulant	EPS mixture	Single foulant	EPS mixture	Single foulant
NaOH	87.3 ± 1.44	84.4 ± 3.33	85.3 ± 1.68	83.2 ± 1.54	92.1 ± 2.63	88.0 ± 4.78	90.9 ± 2.72	87.8 ± 2.71
NaOCl	73.2 ± 5.37	84.9 ± 2.32	79.5 ± 2.4 9	78.2 ± 2.97	88.2 ± 2.87	89.8 ± 1.00	86.8 ± 2.61	87.6 ± 5.63
SDS	64.3 ± 3.31	68.6 ± 4.87	66.7 ± 5.95	66.5 ± 3.88	72.3 ± 7.22	74.9 ± 5.02	74.1 ± 2.69	72.6 ± 4.24
EDTA	79.3 ± 11.7	78.8 ± 4.51	74.6 ± 1.34	73.0 ± 2.06	96.8 ± 7.06	88.1 ± 1.09	87.9 ± 0.86	84.7 ± 2.06
10 mM NaCl	70.8 ± 0.99	84.4 ± 5.51	—	—	79.2 ± 0.37	83.8 ± 2.45	—	—
8.5 mM NaCl & 0.5 mM $CaCl_2$	—	—	69.2 ± 2.07	59.8 ± 4.79	—	—	80.3 ± 0.73	80.1 ± 3.29

Backwash-induced flux recovery using chemical solutions (except SDS) was superior than either electrolyte solutions for BSA-dominant mixture fouled membranes. In addition, each cleaning solution achieved similar flux recovery for BSA-only foulant and BSA-dominant mixture. For alginate-only foulant and alginate-dominant mixture, backwashing was clearly more effective than cross-flow cleaning with the electrolyte solutions (Figure 2.1a,b and 2.3a,b). On the other hand, there was no significant difference between the recovered flux of cross-flow cleaning and backwashing for BSA-only fouled membrane in both electrolyte solutions (Figure 2.1c,d). However, the presence of other components in the BSA-dominant mixture made backwashing

more effective than the cross-flow cleaning, exemplifying the importance of other EPS constituents, such as alginate (here at a 5 wt %), in determining the "stickiness" of the fouled layer, even if their concentrations are relatively low.

To evaluate the impact of alginate and octanoic acid in cleaning the BSA-dominant EPS mixture, NaOH cleaning results of membranes fouled with BSA-only, a BSA-dominant mixture representing EPS from gram-positive bacteria, a mixture without octanoic acid (BSA:alginate:DNA = 65:10:25), and a mixture without alginate (BSA:DNA:octanoic acid = 61.67:21.67:16.66) were compared (Figure 2.5). The flux decline induced by BSA-dominant mixture without octanoic acid was 60.7 ± 4.37 % and 61.7 ± 1.69 %, while for BSA-dominant mixture without alginate the flux decline was 40.6 ± 2.55 % and 60.0 ± 4.59 % for the 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM CaCl2 solutions, respectively (Figure 2.5a, b, gray bars). Thus, as the amount of alginate in the mixture increased, the flux decline was greater, particularly for the BSA-dominant mixture without the octanoic acid, which also had elevated BSA concentrations.

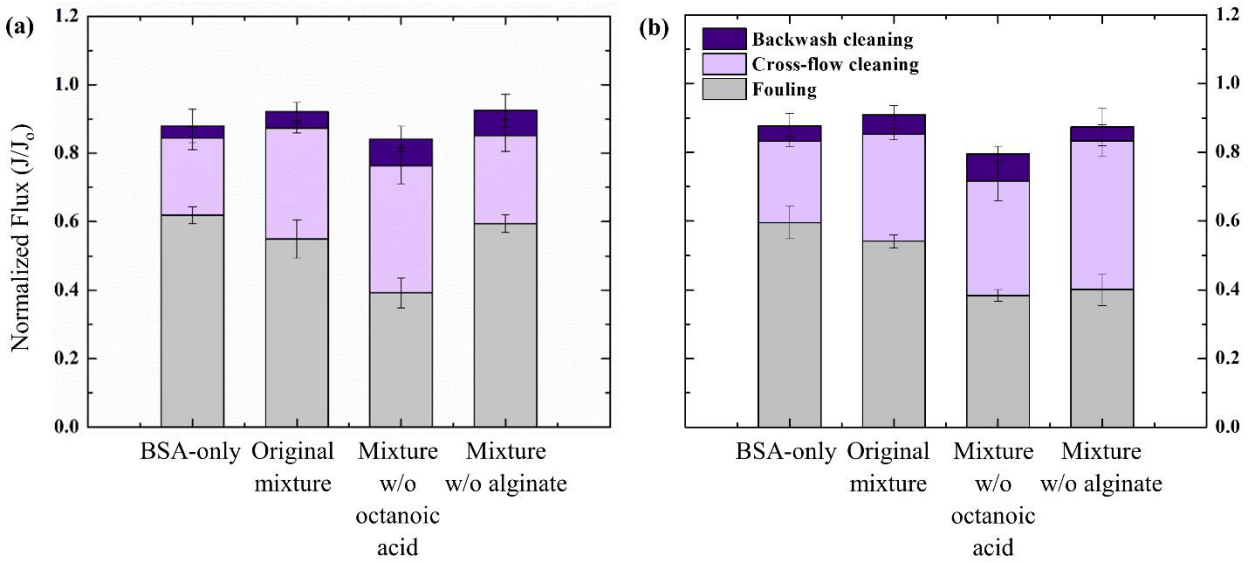

Figure 2.8 Normalized flux of BSA-only fouled membrane and BSA-dominant mixture fouled membranes with a) 10 mM NaCl b) 8.5 mM NaCl and 0.5 mM $CaCl_2$ cleaned by NaOH.

In the presence of 10 mM NaCl, cross-flow cleaning with NaOH achieved similar cleaning performance for the pure BSA, BSA-dominant mixture, and BSA-dominant mixture without alginate, with recovered values being, 84.4 ± 3.33 %, 87.3 ± 1.44 % and 85.1 ± 4.61 %, respectively, (Figure 2.5a, light purple bars). However, when the octanoic acid was removed, washing the membrane with NaOH was only able to recover flux up to 76.3 ± 5.18 %, possibly due to the increased alginate concentration. Backwashing the fouled membranes increased flux by between 4 - 7 % and never achieved full flux recovery under any conditions (Figure 2.5a, dark purple bars). In addition, in the presence of Ca^{2+}, removing octanoic acid or alginate had no significant impact on flux decline or recovery of the fouled membranes (Figure 2.5b). As the data in Figure 2.3 shows, the presence of BSA in the EPS mixture governs the effectiveness of the cleaning process. The data in Figure 2.5 confirms this as none of the BSA-dominant mixtures or pure BSA can be completely removed, regardless of the cleaning agent used or hydrodynamic conditions.

Unlike the single component fouled membranes, where no significant difference was observed between the recovered flux of chemical cleaning agents and electrolyte solutions, the recovery flux was enhanced by the usage of chemical agents (NaOH) for the multi-component synthetic EPS mixtures fouled membranes. However, full recovery was not achieved simply by the addition of chemical agents even during backwashing. Only the synthetic EPS mixtures without BSA could be removed completely from the membrane surface. Thus, we speculate that BSA is the key component that keeps the mixture adhered to the membrane surface, which prevents complete flux recovery. Targeting protein removal from the bacterial EPS may help developing an efficient biofilm management strategy.

Membranes fouled by EPS extracted from *P. aeruginosa* (2.5 mg) in the presence of 8.5 mM NaCl and 0.5 mM $CaCl_2$, showed a flux decline of 47.3 ± 3.95 %, which is closer to the fouled

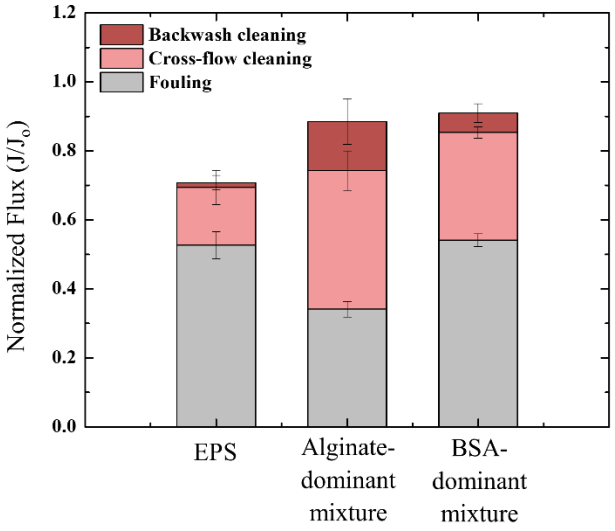

Figure 2.9 Normalized flux of *P.aeruginosa* EPS, alginate-dominant mixture and BSA-dominant mixture fouled membranes with 8.5 mM NaCl and 0.5 mM $CaCl_2$ cleaned by NaOH.

flux of the BSA-dominant mixture (45.9 ± 1.95 %) than the alginate-dominant mixture (65.9 ± 2.23 %) (Figure 2.6). According to Orgad et al. (2011), the polysaccharide/protein ratio in the EPS extracted from *P. aeruginosa* PAO1 wild type strains was 3.1 ± 0.22, [53] which is 4 times smaller than the ratio of the alginate-dominant mixture. This could explain the smaller flux decline of the *P. aeruginosa* EPS fouled membrane compared to the alginate-dominant mixture. However, cleaning the EPS fouled membrane with NaOH was less effective, with the recovered flux only reaching 70.8 ± 2.13 % of its initial value. This is likely because *P. aeruginosa* synthesize 3 wild-type polysaccharides, alginate, Psl, and Pel. Psl is a neutral polysaccharide while Pel a is positively charged polysaccharide at pH below 6.7.[73] Therefore, *P. aeruginosa* EPS is less pH sensitive compared to alginate, and is less likely to be solubilized at elevated pH. This is in contrast to the membranes fouled by the artificial EPS mixtures, which recovered to 88.5 ± 6.64 % and 90.9 ± 2.72 %, for the alginate- and BSA-dominant mixtures. Backwashing the EPS fouled membrane had almost no effect on recovering flux, which is comparable to the backwashing of BSA-dominant mixture fouled membrane, proving that the proteins in the EPS mixture provide integrity to the matrix, corroborating with the results of the BSA-dominant mixture. Despite the use of various chemicals, complete flux recovery was not observed with the EPS or synthetic EPS mixtures. Higher concentrations of these cleaning chemicals, and in particular NaOH and NaOCl, may improve flux recovery. However, studies have

demonstrated that increasing the cleaning agent's concentration may lead to a deterioration of the membrane material itself.[5,7,22] The chemical concentrations used in this paper were recommended by the manufacturer to minimize the damage to the membrane integrity.

2.4 Conclusion

We have investigated the ability of commonly used cleaning chemicals and processes to restore the flux of membranes fouled by individual biofilm components, as well as by mixtures of these components that represent the EPS from gram positive and gram-negative bacteria. Since EPS represents more than 90 % of the biofilm mass, understanding how the different components of the EPS react with these standard cleaning approaches can inform biofilm management approaches in systems ranging from heat exchangers to water treatment membranes. The model biofilms contained a representative mix of a polysaccharide (alginate), protein (BSA), eDNA (from salmon), and lipids (octanoic acid), with the ratios of these individual components depending on whether they were used to model a gram positive or negative biofilm. The degree of flux decline upon membrane fouling was a function of the fouled layer structure, with the gel-like alginate leading to the most severe flux decline, while membranes fouled by the globular BSA experienced the least decline.

Flux recovery was highly dependent on the fouling species, cleaning method, and the species of the background electrolyte. When the membrane was fouled with alginate, flux was partially recovered through cross-flow cleaning, and fully recovered when the membrane was backwashed, likely caused by the strong cohesion of the alginate gel coupled to poor adhesion to the membrane's surface. The presence of cleaning chemicals did not significantly impact flux recovery and this result is in line with other studies where various concentration of NaCl was effective of cleaning organic fouled membrane even in the presence of Ca^{2+}.[35,46] However, in the

presence of Ca^{2+}, complete flux recovery was only achieved when a metal chelating agent (EDTA) was used to backflush the membrane, illustrating the importance of specific foulant-membrane interactions, and in particular, the importance of divalent cations in forming ionic "bridges" between the foulant and the membrane surface. The breakdown of foulant-foulant interactions brought about by Ca^{2+} complexation by EDTA could also facilitate the enhanced removal. When the membrane was fouled with BSA, no cleaning method achieved complete flux recovery, likely because of strong hydrophobic interactions between hydrophobic domains on the BSA molecule and the membrane surface; in this case, the identity of the ionic species had no impact on membrane recovery. Membranes fouled with DNA could be completely recovered, with cleaning agents playing no significant role.

Unlike the case when the membranes were fouled by a single EPS component, where no significant difference was observed when chemical cleaning agents were used to clean the membranes compared to using the background electrolyte (except when alginate was used in the presence of Ca^{2+}, where only the addition of EDTA allowed for complete flux recovery), the effectiveness of flux recovery methods for synthetic EPS mixture was enhanced by the usage of NaOH. Previous studies also found alkaline to be one of the most effective cleaning agents to clean membrane fouled by surface water or waste water.[43–48] However, full recovery was never achieved by the addition of chemical agents. Only the synthetic EPS mixtures without BSA could be completely removed from the membrane surface. Thus, we conclude that the presence of BSA is the key reason that prevents the complete cleaning (and flux restoration) of biofouled membrane surfaces. Specifically targeting EPS proteins, through the use of protease, for example, may allow for the complete cleaning of the membrane surface and the restoration of flux. In addition, the

complete removal of attached proteins could reduce the re-fouling of the membrane surface by completely eliminating the protein conditioning layer.

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Appendix 2A

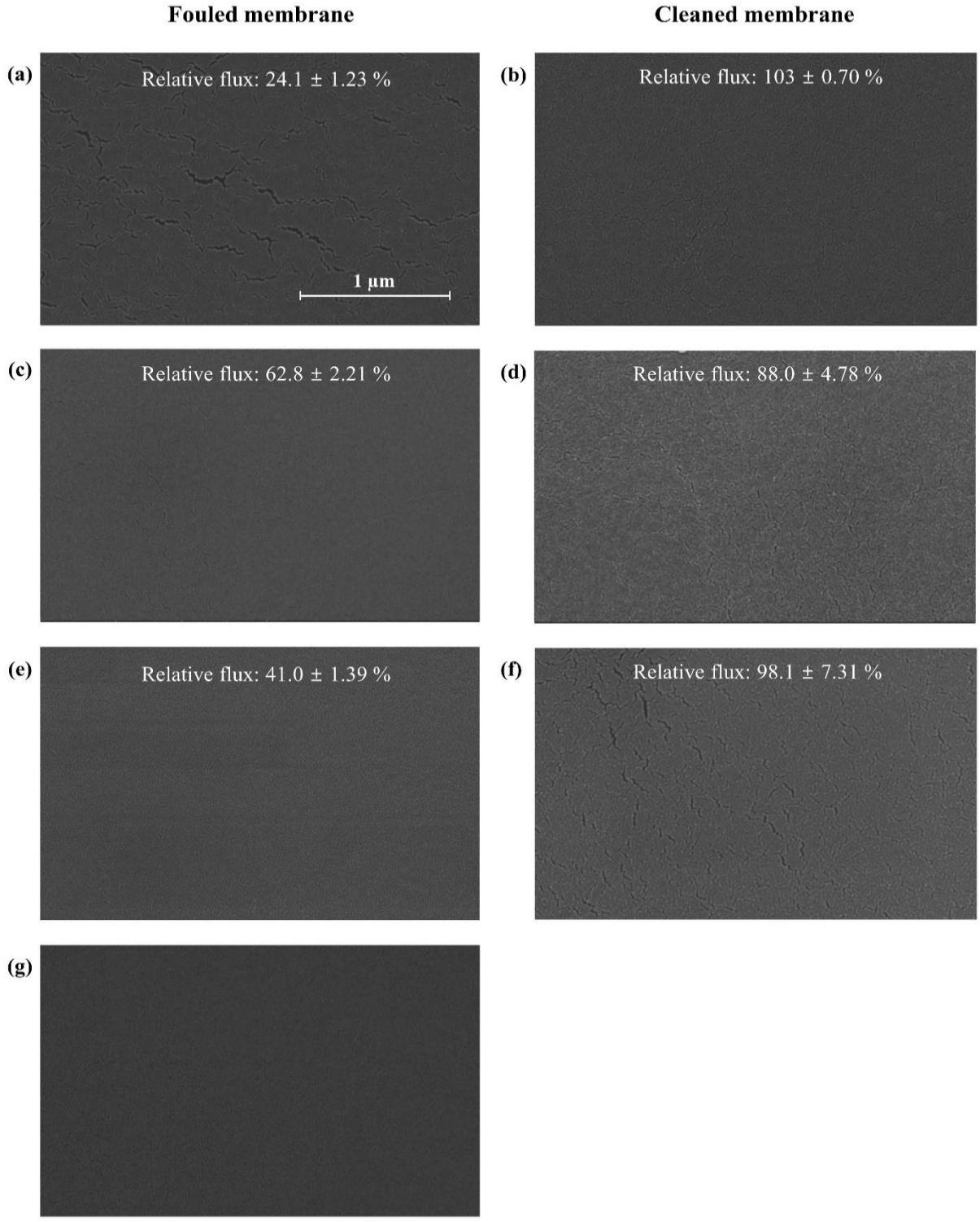

Figure 2A.10 SEM images of membranes fouled with (a) alginate, (c) BSA and (e) DNA in 10 mM NaCl and (g) pristine membrane before fouling. Images of cleaned membrane surfaces after membranes were cleaned (both cross- and backwash) with NaOH for alginate (b), BSA (d), and DNA (f) fouled membranes.

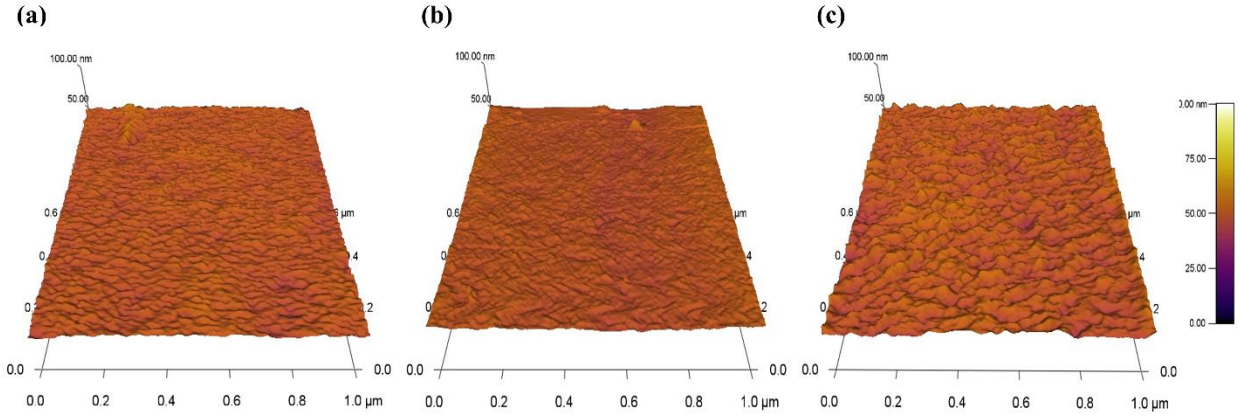

Figure 2A.11 AFM images of (a) pristine membrane, (b) alginate fouled membrane and (c) BSA fouled membrane.

Chapter III.

Impact of Chemical Cleaning Agents on Alginate and BSA Analyzed Using Quartz Crystal Microbalance (QCM)

3.1 Introduction

The high concern for severe water scarcity throughout the world has led to the evolution of water treatment technologies. Over the past few decades, the expansion of filtration membrane technology contributed to its increasing recognition as a reliable process for producing and distributing high quality water to the local community.[1] Despite recent innovative pretreatment strategies for the feed water and modification of the membrane properties, fouling is an evitable problem in membrane filtration.[2] Among potential foulants biofilm is one of the most challenging and prevalent problem in water treatment processes.[3] Especially, extracellular polymeric substances (EPS) in biofilms contribute significantly to the decrease in membrane filtration performance (flux decline and poor salt rejection). Moreover it has greater impact on determining the membrane cleaning efficiency than the microorganisms themselves.[4] Therefore, EPS have traditionally been used as the representative biofouling material and can be characterized its major components, which include polysaccharides and proteins. EPS polysaccharides and proteins are responsible for mediating the adhesion of the microbial microcolonies to surfaces and providing mechanical stability of the biofilm.[5] Generally, polysaccharides are weakly negatively charged due to the presence of uronic acids or ketal-linked pyruvates (containing carboxylic groups), which contributes to their anionic properties.[2] Unlike polysaccharides, proteins usually have amphoteric charge properties due to the presence of both carboxylic ($-COO^-$) and amine ($-NH3^+$) groups. They are positively charged at pH below their respective isoelectric points and become negatively charged above the isoelectric points.[3] Divalent cations, commonly found in natural water, are known to bind to carboxylic groups associated in macromolecules, such as polysaccharides and proteins, to partially neutralizes their negative charges.[7] In addition, divalent cations can bind

neighboring macromolecules together and bridge macromolecules to a surface to form an extensive cross-linked gel structure, which enhances membrane fouling.[8]

Quartz-crystal microbalance (QCM) is an arising technology to biofilm because of its capability to observe mass being absorb onto and removed from the quartz sensors in real time.[9] Quartz is a piezoelectric material, in which application of voltage results in mechanical deformation of the material. Applying alternating voltage leads to a cyclical deformation resulting in an oscillatory motion. When additional mass adsorbs onto the substrate, the resonance frequency of oscillation decreases.[10] The frequency change is proportional to the mass of the adsorbed material, which can be calculated by using Sauerbrey equation[11]:

$$\Delta m = -C_{QCM}\frac{\Delta f_n}{n}$$

where, Δm is the absorbed mass, C_{QCM} is the mass sensitivity constant (17.7 ng cm^{-2} Hz $^{-1}$), n is the $n$the overtone, and Δf_n is the change in resonance frequency.

Numerous studies have demonstrated QCM to be a powerful tool in studying organic, EPS or bacterial adhesion and different stages of biofilm formation.[11–14] For example, Gutman et al. (2014) studied the adsorption of model bacteria including *Escherichia coli*, and *Pseudomonas aeruginosa* and vesicles made of bacterial lipopolysaccharides and glycosphingolipids on QCM sensors.[15] Easily modified quartz crystal sensors allow to investigate the interaction between foulants and various surfaces. In another study, adsorption of alginate and BSA was investigated on self-assembled monolayers with seven different ending chemical functionalities (−CH3,

−O−phenyl, −NH2, ethylene-glycol, −COOH, −CONH2, and −OH), which was formed on top of the gold-coated QCM-D sensor.[16]

The complexity of the biofilm makes it difficult to examine the biofouling behavior, which is depended on multiple factors including the feed water composition, membrane surface properties, foulant-membrane and foulant-foulant interactions.[4] However, any insights to the adherence of EPS component on membrane surface and its removal through interactions with chemical cleaning agents may provide critical knowledge to effectively remove the biofilm and develop a sustainable biofilm management strategy. In this study, QCM was used to study the model EPS component adhesion to PES (polyethersulfone)-coated sensor mimicking PES membrane surface and its detachment using cleaning agents. The QCM results were compared to fouling and cleaning experiments conducted in a flow cell unit with PES ultrafiltration (UF) membrane from previous research (Chapter 2) to better understand the interactions between EPS components, PES-coated surface and the chemical cleaning agents.

3.2 Experimental Section

3.2.1 Organic Foulants and Background Solution

To represent EPS polysaccharide and protein, alginate (1000 cp, 1% in water) and BSA (66.5 kDa) was chosen as model foulants. Alginate and BSA were purchased from Sigma-Aldrich (St. Louis, MO) and were received in a powder form. The foulants were well mixed and freshly prepared in two background solutions: 100 mM NaCl or 85 mM NaCl with 5 mM $CaCl_2$, filtered with a 0.45 µm hydrophilic filters (Millipore, Billerica, MA). Initially, the total ionic strength was 10 mM to represent the lower limit of groundwater and the upper limit of surface water. However, the small frequency change detected by the QCM sensor indicated poor adhesion of the foulants

onto the surface at low ionic strength. Thus, to ensure sufficient amount of foulant is absorbed on the QCM sensor surface, the total ionic strength of the background solutions was increased ten times (100 mM).

3.2.2 Chemical Cleaning Agents

Two types of chemical cleaning agents were used in this study, which are most commonly used in the industry for organic and bio-fouled membranes: A metal chelating agent (ethylene diamine tetra acetic acid (EDTA)) and an oxidizer (NaOCl). The efficacy of EDTA to remove organic foulant was tested at two different pHs (pH 7 & 11), which were adjusted by NaOH. On the other hand, to test the effect of chemical agent's concentration on foulant removal, three different concentrations (5, 100, 250ppm) of NaOCl were evaluated. Each stock chemical agents were prepared freshly by dissolving each chemical in deionized (DI) water without further purification.

3.2.3 Polyethersulfone (PES)-coated QCM Sensor

A bare gold QCM sensor was coated with PES solution to mimic PES membrane surface. The coating solution was prepared by dissolving 0.6 wt% PES in dichloromethane (DCM) and filtering through 0.45 μm hydrophilic filters (Millipore, Billerica, MA). The solution was spin-coated onto the bare gold sensor at 2700 rpm for 1 minute. After each experiment, the PES layer and any remaining organic foulant was removed by soaking the PES-coated sensor in a 5:1:1 mixture of DI water, ammonia (25 %) and hydrogen peroxide (30 %). The solution was heated for 5 minutes at 75 °C. Then the sensor was thoroughly rinsed with DI water followed by N_2 gas drying, 99% ethanol rinsing and final N_2 gas drying.

3.2.4 Deposition and Cleaning Experiments on PES-Coated Sensor in QCM

The deposition and cleaning experiments of EPS model components on PES-coated gold sensor were analyzed in QCM-D E4 system (Q-Sense, Gothenburg, Sweden), which measures the change of the oscillation frequency exerted on the PES-coated sensor during a parallel 150 µL/min flow of the aqueous solution at constant temperature (22 °C). The variations of frequency shift (Δf, Hz) was measured for five overtones (n = 3, 5, 7, 9 and 11) and the 9th overtone is presented in the results section. Before each measurement, the sensors were rinsed thoroughly with DI water and dried with pure N_2 gas. Either 100 mg/L of alginate or BSA dissolved in the background solution was used as the EPS fouling solution.

The EPS fouling solutions or cleaning agents were injected sequentially to the QCM flow cell in the following order after acquiring a stable baseline with DI water overnight: (1) DI water baseline for 30 min; (2) conditioning with background solution (BS) (100 mM NaCl or 85 mM NaCl with 5 mM $CaCl_2$) for 30 min; (3) fouling with 100 mg/L alginate or BSA solution for 60 min; (4) stabilizing adsorbed foulant layer with background solution (BS) for 40 min; (5) cleaning with chemical agent (EDTA or NaOCl) for 40 min, (6) Removing excess cleaning agent and stabilizing remaining foulant layer with background solution (BS) for 40 min; and lastly (7) cleaning with DI water for 30 min to resemble pure water flux after cleaning in a UF flow cell unit (Figure 3.1A).

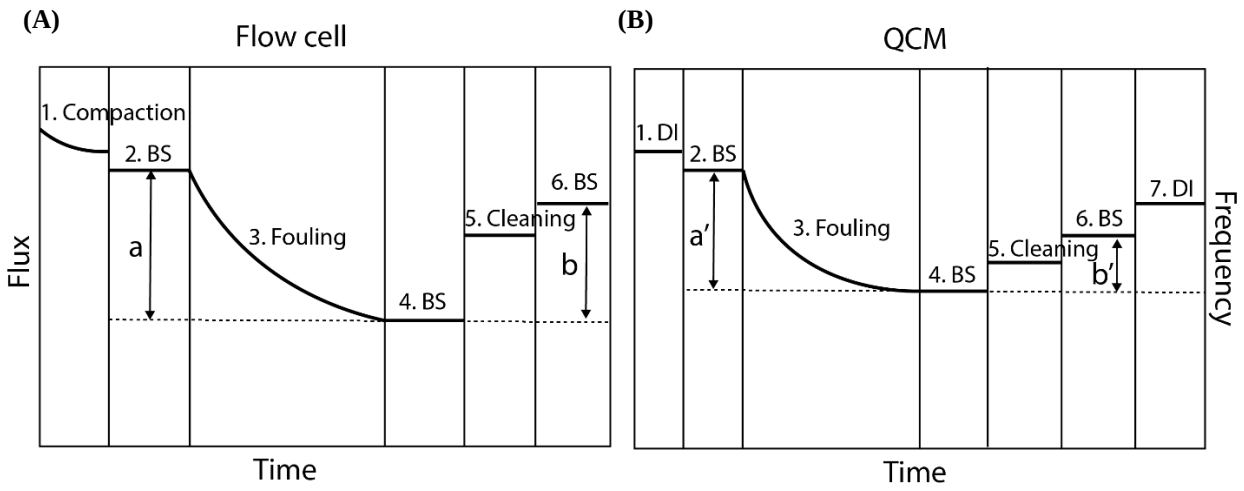

Figure 3.12 Schematic diagram of the membrane fouling and cleaning experiments in (A) UF flow cell and (B) QCM

Typically, the cleaning efficiency is calculated based on the frequency shift after DI water injection instead of background solution to mimic the change in pure water flux after cleaning in a filtration flow cell unit. However, in previous experiment (Chapter 2) the flux recovery was evaluated based on the final and initial water flux measured with the background solution not DI water. Therefore, to draw a comparable conclusion from the UF flow cell and QCM results, frequency changes after background solution cleaning was used to calculate the cleaning efficiency (Step 2&6 in Figure 3.1). For QCM experiment, the cleaning efficiency was calculated as $\frac{b}{a} \times 100\%$ (Figure 3.1A), while for the UF flow cell experiment it was $\frac{b\prime}{a\prime} \times 100\%$ (Figure 3.1B).

3.3 Results and Discussion

3.3.1 Characteristics of PES-coated QCM Sensor

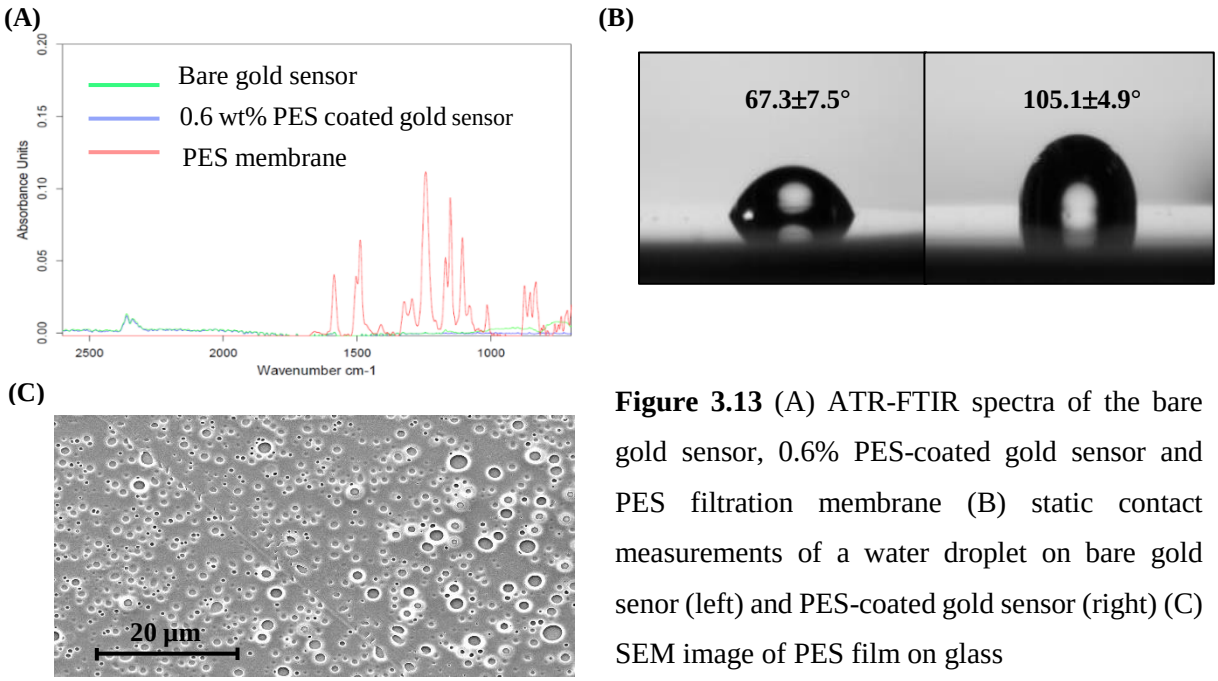

Figure 3.13 (A) ATR-FTIR spectra of the bare gold sensor, 0.6% PES-coated gold sensor and PES filtration membrane (B) static contact measurements of a water droplet on bare gold senor (left) and PES-coated gold sensor (right) (C) SEM image of PES film on glass

To mimic the surface chemistry of a PES membrane, QCM gold sensors were spin-coated with 0.6 wt% PES in DCM. According to the ATR-FTIR results, the spectrum of the PES-coated sensor was almost identical to the bare gold sensor but significantly different from an actual PES membrane (Figure 3.2A). We speculate that the PES layer on the sensor was too thin for the instrument to detect similar peak intensities from the PES membrane. However, the contact angle measurement and the SEM image confirmed that the PES layer existed on top of the gold sensor (Figure 3.2B,C). The contact angle of a water droplet on the bare gold sensor was 67 ± 7.5˚, while on the PES-coated gold sensor it was 105.1 ± 4.9˚. The PES layer made the surface more hydrophobic, which is consistent with the findings in the literature (PES membrane contact angle: 100.6±2.1°).[17] The SEM image of the PES film revealed an uneven surface coated on glass, most likely due to the fast evaporation of DCM (solvent) during spin-coating.

3.3.2 Influence of Calcium on Alginate and BSA Deposition

Divalent ions, such as calcium, have a major effect on irreversible organic fouling or biofouling in membrane filtration. To test the effect of calcium on deposition of polysaccharide and protein, two model EPS components (alginate and BSA) dissolved in two background solutions (100 mM NaCl and 85 mM NaCl with 5 mM $CaCl_2$) were deposited onto the PES-coated sensors.

The frequency shifts caused by the deposition of alginate in the presence and absence of calcium were slightly different. In the presence of calcium, the frequency shift from the baseline was 9.69 ± 2.36 Hz, while in the absence of calcium, the frequency shift was 8.37 ± 1.77 Hz. This indicates that the amount of alginate attached to the PES-coated gold sensor was greater in the presence of calcium most likely due to the gel formation. Numerous studies observed calcium to bind neighboring alginate molecules as well as between alginate and the negatively charged surface to form either a globular structure or a dense gel layer on the surface.[18]

The observed frequency shifts caused by BSA deposition were 17.95 and 20.88 with the background solution of 100 mM of NaCl and 85 mM NaCl with 5 mM $CaCl_2$, respectively. This data may not be as reliable as the alginate deposition since insufficient amount of data was collected from experiments conducted with BSA. However, in spite of the lack of data, it should be noted that calcium promoted deposition of BSA, which corroborates with other studies that showed enhanced membrane fouling by BSA at higher concentration of calcium.[19] In addition, the frequency shift caused by the deposition of BSA was about two times greater than the frequency shift caused by alginate deposition, despite the fact that PES-coated sensors were exposed to the same mass concentration of foulants over the same time period (60 minutes). This result implies

that BSA adheres to or interacts with the PES layer more strongly than alginate and the presence of protein in the feed may influence the formation of a condition layer and fouling behavior

Recent studies and previous work (Chapter 2) demonstrated that the permeate flux decline by the alginate fouled membrane was more severe than BSA fouled membrane,[19,20] which may seem to contradict the results discovered in this QCM experiment. However, Contreras et al. (2011) discovered that the intial adsorption rate of BSA was higher than alginate during the first 2 hr and once equilibrium was achieved the adsorbed mass of algiante was greater than BSA.[16] This could explain the greater frequency shift of BSA obsevered in this experiment since the deposition of foulants was carried out for only 40 min.

3.3.3 Cleaning Efficiency of a Chelating Agent on Alginate

Once alginate was deposited onto the PES-coated sensors, the alginate was exposed to 40 minutes of cross-flow cleaning with EDTA, a metal-chelating agent, at pH 11 and pH 7 (adjusted by NaOH). Metal chelating agents are known to replace metal ions, especially divalent cations, by ligand exchange reactions, which removes the intermolecular bridges within the EPS matrix as well as those between the biofilm and the surface.[21] The frequency recovery after EDTA cleaning was higher at pH 11 than at pH 7 and more pronounced with calcium present in the background solution (Figure 3.3A), indicating that at these conditions the removal of alginate is higher. Based on the method described in the experimental section, the cleaning efficacy of EDTA on alginate was calculated, which was 57.7 ± 0.22 % and 69.2 ± 2.94 % at pH 11, 23.6 ± 3.45 % and 33.0 % at pH 7 in the presence of 100 mM NaCl solution and 85 mM NaCl with 5 mM $CaCl_2$ solutions, respectively (Figure 3.3B).

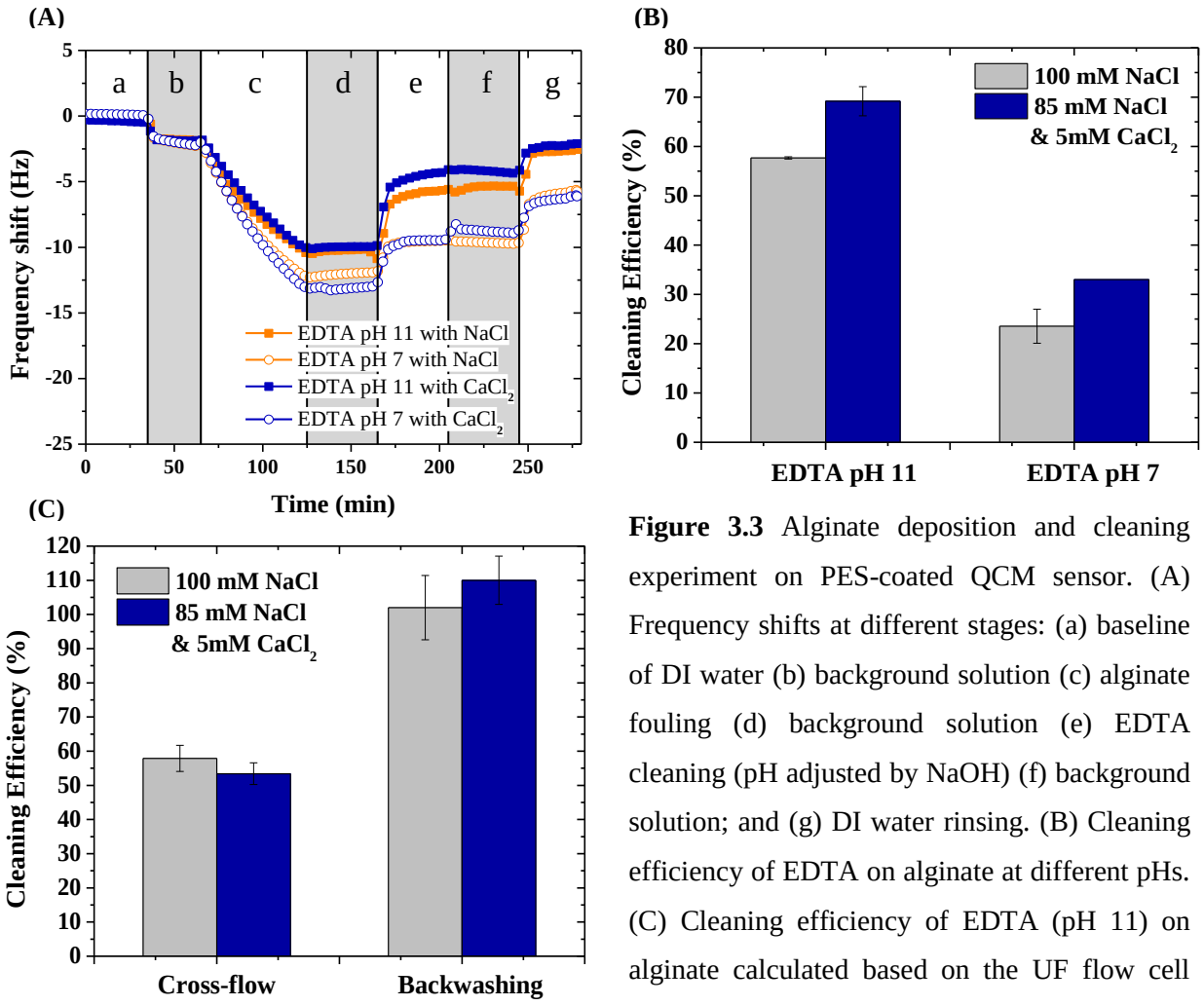

Figure 3.3 Alginate deposition and cleaning experiment on PES-coated QCM sensor. (A) Frequency shifts at different stages: (a) baseline of DI water (b) background solution (c) alginate fouling (d) background solution (e) EDTA cleaning (pH adjusted by NaOH) (f) background solution; and (g) DI water rinsing. (B) Cleaning efficiency of EDTA on alginate at different pHs. (C) Cleaning efficiency of EDTA (pH 11) on alginate calculated based on the UF flow cell experiment from Chapter 2.

As expected, the cleaning efficiency of EDTA at pH 11 was twice as greater than at pH 7 for both background solutions. At pH 11.0, all the carboxyl and amino groups of EDTA are fully deprotonated[22] and the ability of EDTA to perform ligand-exchange reaction with the gel layer formed by alginate–calcium complexes is increased than pH 7. Consequently, the intermolecular bridges within the gel layer and between the gel layer and the surface are broken down more easily, thus, resulted in a higher cleaning efficiency. The larger quantity of hydroxide ions, which are known to assist hydrolysis of polysaccharide and proteins into smaller molecules[23], at pH 11 contributed to the higher cleaning efficiency as well. Once EDTA loosens the gel layer, NaOH can

hydrolyze the alginate more effectively, which explains the alginate fouling solution dissolved in NaCl and $CaCl_2$ to exhibit greater cleaning efficiency than alginate fouling solution dissolved only in NaCl.

However, complete removal of alginate was not observed with EDTA cleaning, which is consistent with the results found in previous study (Chapter 2). During cross-flow cleaning in the UF flow cell unit, the cleaning efficiency of EDTA at pH 11 was 57.9 ± 3.83 % and 53.4 ± 3.18 % for the alginate fouled membranes in the presence of 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively (Figure 3.3C). In NaCl solution only, both QCM and flow cell experiment showed comparable results of cleaning alginate with EDTA. However, in the presence of calcium, EDTA was 15.8 % less effective removing alginate in the UF flow cell than QCM and only when alginate was exposed to EDTA through backwashing, 100% cleaning efficiency was achieved. Although similar trend was observed in NaCl background solution, cross-cleaning in the QCM was more effective than in the UF flow cell in the presence of calcium despite of greater mass of alginate was absorbed onto the QCM surface than the UF membrane. The estimated mass of alginate deposited on the QCM surface is 0.39 mg/cm^2 and on the UF membrane is 0.21 mg/cm^2. This suggests that comparing results of the two cases might not be feasible.

3.3.4 Cleaning Efficiency of an Oxidizer on Alginate

Another effective cleaning agent for removing biofilm is an oxidizer. It is known to kill microorganisms and oxidize functional groups in organic and biological foulants, which reduces the adhesion between foulants and membrane surface.[21]

NaOCl cleaning was performed using various concentrations: 250 ppm, 100 ppm and 5 ppm (Figure 3.4A). The cleaning efficiency of NaOCl at concentration of 250ppm was 162.7 ±

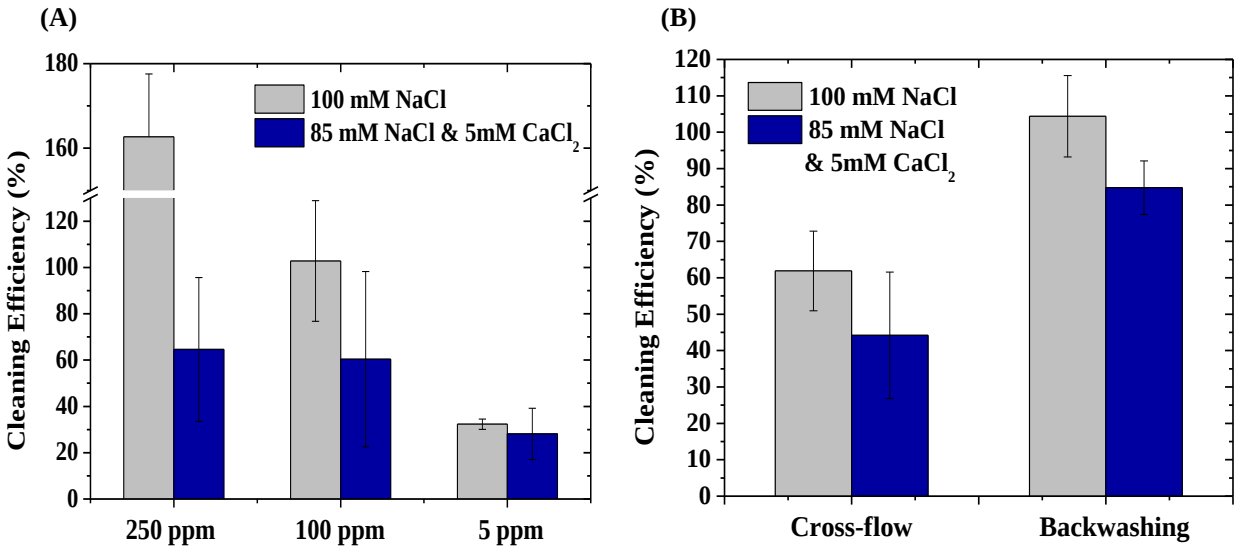

Figure 3.15 (A) Cleaning efficiency of NaOCl on alginate at different concentrations measured by QCM (B) UF flow cell

14.9 % and 64.6 ± 31.1 %, at concentration of 100ppm it was 102.8 ± 26.1 % and 60.3 ± 37.9 % and at concentration of 5ppm it was 32.3 ± 2.20 % and 28.1 ± 11.1 % on the alginate absorbed sensors in the presence of 100 mM NaCl solution and 85 mM NaCl and 5 mM $CaCl_2$ solutions, respectively. Surprising, in the presence of NaCl only, the cleaning efficiency exceeded 100% at NaOCl concentrations of 250 ppm and 100 ppm. It is speculated that the indiscriminating attack of the NaOCl on chemical bonds removed the alginate along with the PES layer coated on the QCM sensor during the cleaning process, which contributed to the frequency shift higher than the initial baseline, leading to cleaning efficiency over 100%. However, cleaning with 5ppm NaOCl had little effect on removing alginate regardless of the absence or presence of calcium, indicating that the concentration of NaOCl was too low. In general, the cleaning efficiency of NaOCl was greater in the absence of calcium at all three concentrations possibly because NaOCl could not effectively dissolve the alginate-calcium complex.

Unlike the QCM experiment, only one concentration of NaOCl was tested on the fouled membrane in the flow cell experiment. During cross-flow cleaning, the cleaning efficiency of 100 ppm NaOCl was 61.9 ± 10.93 % and 44.2 ± 17.41 % for the alginate fouled membranes in the presence of 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively (Figure 3.4B). The cleaning efficiency of NaOCl measured from QCM was noticeably higher than cross-flow cleaning but similar to backwashing in the UF flow cell. Backwashing with 100 ppm NaOCl yielded cleaning efficiency of 104.4 ± 11.2 % and 84.7 ± 7.36 % for the alginate fouled membranes in the presence of 10 mM NaCl solution and 8.5 mM NaCl and 0.5 mM $CaCl_2$ solutions, respectively. Again, this illustrates that cross-flow cleaning in the QCM is more effective than UF flow cell since the mass of alginate adsorbed on the QCM sensor was twice the amount on the UF membrane yet the cleaning efficiency of NaOCl is higher in the QCM.

3.3.5 Cleaning Efficiency of Chelating Agent on BSA

The effect of EDTA at pH 11 on BSA deposited on the PES-coated sensor was evaluated in the same manner as the alginate cleaning process. The cleaning efficiency of EDTA at pH 11 was 11.7 % and 7.69 % for the BSA absorbed sensors, in the presence of 100 mM NaCl solution and 85 mM NaCl with 5 mM $CaCl_2$ solutions, respectively (Figure 3.5A). Compared to alginate removal, cleaning with EDTA yielded poor results with BSA removal from the PES-coated sensors, especially in the presence calcium. Similar trend was observed in cross-flow cleaning of the BSA fouled membrane in the flow cell (Chapter 2). The cleaning efficiency of EDTA at pH 11 on BSA fouled membrane was 45.1 ± 5.63 % and 30.6 ± 6.16 % in the presence of 10 mM NaCl solution and 8.5 mM NaCl with 0.5 mM $CaCl_2$ solutions, respectively (Figure 3.5B).

This confirms that the presence of calcium, results in more favorable deposition of BSA molecules onto a surface due to the reduction of the negative charges among the BSA molecules

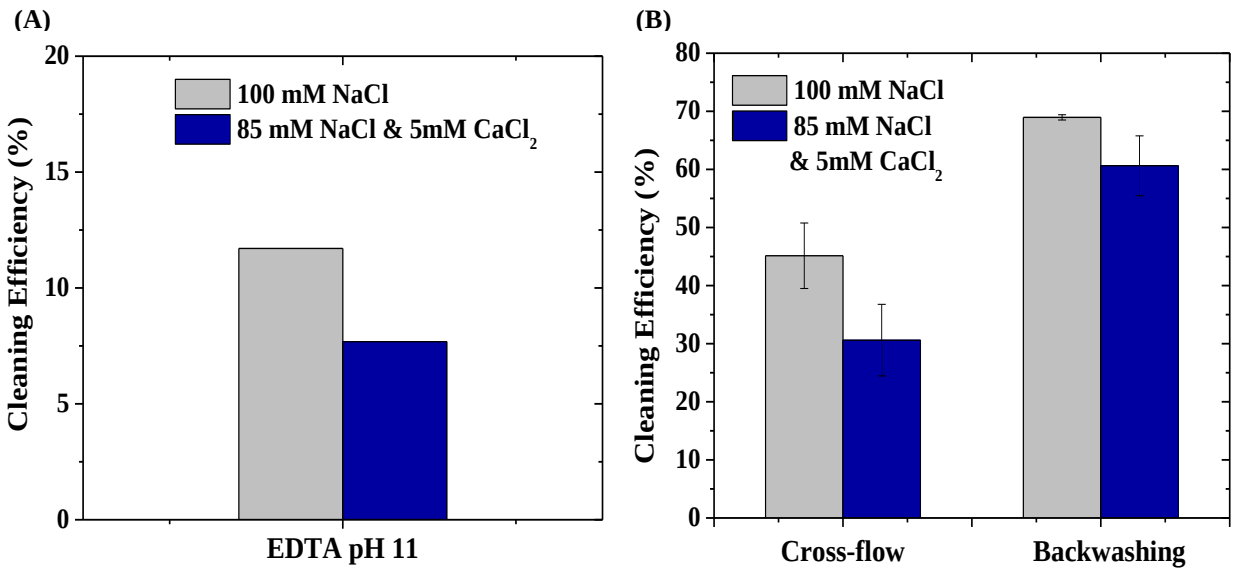

Figure 3.16 Cleaning efficiency of EDTA on BSA at pH 11 measured by (A) QCM (B) UF flow cell

and between the BSA molecules and the surface.[19] In addition, this illustrates the difference between BSA and alginate fouling. Even though alginate forms a denser layer in the presence of calcium, the rigid structure of the BSA is more tightly bound to surface, contributing to irreversible fouling and ineffective cleaning than alginate. This supports our discovery from previous work (Chapter 2), which showed that the presence of BSA is the key reason that prevents the complete cleaning of biofouled membrane surfaces. Nevertheless, it should be noted that cross-flow cleaning of BSA deposited surface with EDTA in the UF flow cell was greater than QCM, which is opposite to the results from alginate cleaning. Cross-flow cleaning alginate deposited surface with either EDTA or NaOCl exhibited higher cleaning efficiency in QCM than UF flow cell, which implies that direct comparison between the QCM and UF flow cell experiments may not be suitable.

3.3.6 Two Different QCM, Two Different Results

Initially, the QCM experiment was conducted at Ben-Gurion University, Zuckerberg Institute for Water Research in Israel under the supervision of Professor Herzberg. Due to the time constraint of the project, insufficient amount of data was collected and therefore, experiments were

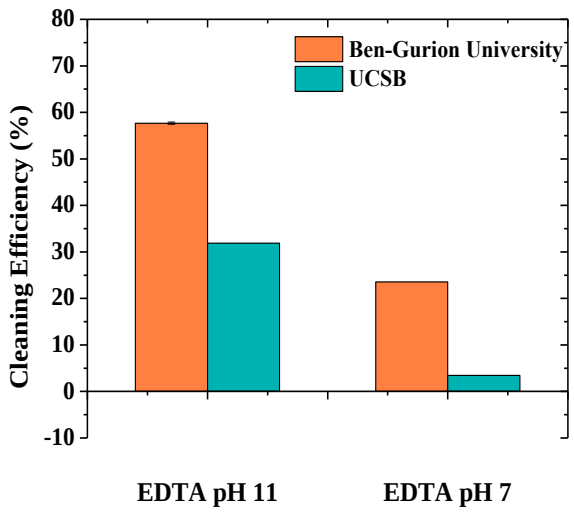

Figure 3.17 Comparison of QCM experiment results conducted at Ben-Gurion University and University of California at Santa Barbara (UCSB). Cleaning efficiency of EDTA on alginate with 100 mM NaCl at different pHs

further carried out at University of California in Santa Barbara (UCSB), Materials Research Laboratory. However, soon it was realized the data collected from both universities were significantly different. (Figure 3.6). Results from UCSB showed that the cleaning efficiency of EDTA on alginate absorbed QCM surface was 26 – 28 % lower than the results collected from Ben-Gurion University at both pHs. The different results may have been caused by several factors. First, the PES pellets and alginate were purchase from different manufactures. Same chemicals purchased from Israel could not be found in the U.S.A. and lack of information given by the manufacturer made it even harder to find suitable replacements. The different chemical properties of the alginates may have changed the fouling behavior and its interaction with EDTA, contributing to the difference in cleaning efficiencies. In addition to using different PES pellets, applying different organic solutions (DCM and Dimethylformamide (DMF)) to dissolve the PES pellets may have altered the PES layer characteristics as well. Therefore, further experiment was not carried out and the project was discontinued.

3.4 Conclusion

In this chapter, we investigated the adherence of model EPS components on PES-coated QCM sensor mimicking PES membrane surfaces and evaluated efficiency of cleaning agents for removing the model EPS components. Furthermore, the frequency shifts in the QCM caused by

the deposition and cleaning of the model EPS component were compared to the flux changes in a UF flow cell observed from previous work (Chapter 2). Alginate and BSA, representing polysaccharide and protein in the EPS, were dissolved in two background solutions (NaCl only and NaCl with $CaCl_2$) and removed from the QCM surface by two chemical cleaning agents: EDTA (metal-chelating agent) and NaOCl (oxidizer). Larger frequency shift was detected by BSA deposition, indicating the adhesion of BSA on PES layer was greater than alginate, especially in the presence of calcium. QCM experiments revealed that cross-flow cleaning with EDTA was preferable removing alginate dissolved in calcium and when calcium was absent in the background solution NaOCl was favorable. Despite the use of the cleaning agent, BSA removal was significantly less than alginate, which is similar to the results observed in the UF flow cell experiment. However, the adsorption of the alginate or the BSA on the QCM showed different outcomes to the flux decline in the UF flow cell. Therefore, the extent of adsorption on the QCM is not reliable to predict the extent of membrane fouling. Moreover, inconsistent results regarding the relationship between the amount desorbed from the sensor and flux recovery observed after membrane cleaning also prove that cleaning efficiency is not directly comparable for both systems. However, both QCM and UF flow cell experiment demonstrate that removing BSA is challenging and suggest focusing on protein removal to develop an effective biofilm management strategy.

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Chapter IV.

Conclusion

This dissertation focuses on understanding how biofilm reacts to specific cleaning agent and determining the key component of the biofilm that affects the biofilm removal. In Chapter 2, a homogeneous layer of a single biofilm component (polysaccharides, proteins, nucleic acids) was studied on an ultrafiltration membrane in a 3D-printed flow cell and its interaction with different cleaning solutions (base, oxidizer, surfactant, chelating agent) was evaluated by comparing permeate flux. The alkalis and oxidizer showed the best performance for removing the individual components. However, the presence of calcium in the feed solution hindered the ability of the cleaning solutions to completely remove the foulants in the absence of a chelating agent. Backwashing the membranes fouled with polysaccharides and DNA resulted in full flux recovery but had little effect on recovering the flux of protein fouled membranes. Furthermore, the efficacy of cleaning agents towards model EPS mixtures designed to mimic gram-negative and gram-positive bacterial biofilms were tested. As expected, flux recovery was enhanced by the usage of a base and oxidizer. However, full recovery was never achieved using chemical cleaning agents. It was determined that the presence of proteins in biofilms determines their susceptibility to cleaning.

The same experimental approach of Chapter 2 was applied in Chapter 3 but instead of using an UF flow cell the experiments were conducted with a QCM. In Chapter 3, two main components in EPS, polysaccharides and proteins, were deposited on the QCM sensors and cleaned with a chelating agent and an oxidizer. Despite the same fouling duration, a greater mass of proteins was absorbed onto the QCM sensor than polysaccharide. As seen in Chapter 2, the presence of calcium in the feed solution hindered the cleaning efficiency of the chemical agents to remove either the polysaccharide or the proteins, except when a chelating agent at high pH was used to remove the polysaccharide. Although comparison of the QCM adsorption/desorption and UF membrane

fouling/cleaning are not feasible, results from both Chapter 2 and 3 suggests that proteins are the key component for governing the fouling and cleaning of biofilm. Future research should target removing proteins in the biofilm, for example, using protease, which are known to effectively break down the proteins into smaller molecules and investigate the influence on biofilm removal.